Risk assessment of public health hazards covered by visual inspection of swine meat: development of a visual-only meat inspection system for heavy pigs together with a tool for the evaluation of its performances

con il patrocinio

LXVII CONVEGNO NAZIONALE S.I.S.Vet

SOCIETÀ ITALIANA DELLE SCIENZE VETERINARIE

ABSTRACTS

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2° CONVEGNO REEV
RÉSEAU DES ÉTABLISSEMENTS
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CENTRO CONGRESSI
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TERATOGENIC AND DEVELOPMENTAL EFFECTS OF ENROFLOXACIN IN EMBRYOS OF XENOPUS LAEVIS AND OF BRACHIDANIO RERIO.

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Enrofloxacin (EFX) is an antibacterial, belonging to the fluoroquinolones group, widely used in veterinary medicine, in particular for avian mass treatments. The main metabolite of enrofloxacin, ciprofloxacin (CPX, antimicrobial used in human medicine) is still characterized by a strong biological activity. The occurrence of these compounds in superficial waters has been proved by several studies and, although the common concentrations of these pharmaceuticals in freshwaters are in the order of hundred of nanograms per liter[1], peaks of tens milligrams per liter have also been occasionally found [2].
The aim of this study was to assess the teratogenic and developmental effects of EFX and his main metabolite CPX in two model organisms representative of the freshwater compartment: the frog Xenopus laevi and the fish Brachidanio rerio.

Tests were performed following the FETAX (Frog Embryo Assay Xenopus, ASTM, 1998) and FET (Fish Embryo Toxicity Test, OECD, 2012) guidelines [3;4], with minor modifications.

In both organisms no developmental or teratogenic effects were observed when they were exposed to the limit concentration of 100 mg/L of CPX. A significative reduction of tail movements frequency was instead observed in B.rerio embryos exposed to 100 mg/L of EFX. The same compound showed teratogenicity (optic and thoracic edemas) in X.laevis at concentrations of 50 mg/L and higher.

To our knowledge, teratogenic effects of EFX have not been reported yet. While the tested effective concentration are far higher than those usually detected in the freshwater compartment, a possible risk for aquatic organisms cannot be excluded, the different sensitivity of the various species and the occasional peak concentrations of the compound considered.

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[3] ASTM E1439-98: Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) (10 June 1998)
[4] OECD GUIDELINE FOR THE TESTING OF CHEMICALS. DRAFT PROPOSAL FOR A NEW GUIDELINE. Fish Embryo Toxicity (FET) Test.

MICROBIOLOGICAL QUALITY OF WELL WATER OF SWINE FARMS: ONE YEAR SURVEY

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Water is an essential nutrient for livestock and farmers frequently use groundwater for animal watering. While the quality of water for human consuption is strictly regulated no specific requirements exist regarding biological, chemical and physical parameters of the water for livestock supply [1].
The aim of this study was to conduct a baseline study evaluating some microbiological parameters of water for livestock supply.

The survey, granted by Padua University (CPDA 113807-2011) conducted in Veneto Region, involved 20 swine farms. Water was sampled at the closest point to the well (point A) and at the end of the pipeline at the nipples (point C) two times per year (summer and the winter season for a total of 80 samples). Total bacterial count at 22°C and at 37°C, presence and enumeration of Enterococcus spp. and E. coli was performed applying the ISO methods (respectively 6222, 7899 and 9308). [1]. To detect Campylobacter, 2 litres of water were divided into 2 aliquots and processed in parallel by culture and by real-time PCR. Isolation of Campylobacter was performed according to the ISO17955 procedure, slightly modified. After concentration through 0.2 µm pore size membrane filters, DNA extracts were tested by a real-time PCR assay designed to detect simultaneously C. jejuni and C. coli following the protocol described by Toplak et al. [2].

The total bacterial count at 37°C and at 22°C measured at the point A were almost always lower than the threshold values established for drinking water (respectively <20 ufc/ml and <100 ufc/ml). Conversely, at the point C it was frequently high and greater in summer samples vs the winter ones. Only one sample from point A reported the presence of Enterococcus spp. and E. coli in both seasons. Campylobacter was detected at low rate in both samplings, with a higher prevalence during winter months and at real-time PCR assay. Both C. coli and C. jejuni were identified.

These preliminary results demonstrated a high microbiological quality of water of pig farms sampled at point A. The higher contamination detected at the point C could be due to the age of the plumbing of the farms investigated. Real-time PCR revealed to be a very sensitive and reliable method in detecting Campylobacter in water samples. Data obtained would allow the evaluation of the role of microbial population on dissolved drugs bioavailability. These results, together with those of the chemical physical evaluation, will contribute to understand the role of quality of watering water for therapeutic pourposes.

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CHEMICAL AND PHYSICAL QUALITY OF WELL WATER OF PIG FARMS; ONE YEAR OF SURVEILLANCE

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Water represents a fundamental source of life for humans and animals, but while the quality of drinking water for human use is strictly regulated (1), no specific requirements of biological, chemical and physical parameters for animal water are required nowadays.
Animals are able to ingest a wide variety of different types of water and survive, however, some salts and elements, at high levels, may reduce animal growth and production or may cause illness and death. The water quality and availability can play an important role on the animal health and welfare and heavily influence the quality and the safety of food of animal origin.

The research project, presented, granted by Ateneo di Padova (2), was aimed to evaluate the physical, chemical and microbiological properties of water for animal beverage, sampled from pig farms located in the Veneto region, during summer and winter 2012.
Around 120 samples were collected at different points of the distribution systems in 20 pig farms located in Padova. Treviso, Venezia, Vicenza and Verona provinces. Every sample was evaluated focusing on water hardness, ammonia content and organic and inorganic oxygen demand (3). Cationic and anionic species (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, Ti, V, P, S, Sn, Hg, Zn, Cl-, NO3-, NO2-, PO43-, SO42-) were quantified thanks to inductively coupled plasma (ICP) and ionic chromatography techniques.

The preliminary results were encouraging showing that the quality profile of water sampled from farm wells is of comparable good quality of tap water intended for human use.

The final aim of this work will be the correlation between physical-chemical properties and solubility-stability of main antibacterial drugs, usually delivered to the farm animals via medicated water. The parameters reported could influence drug properties such as dissolution rate, solubility and stability; i.e: some dissolved metals can modify antibacterial bioavailability, thus affecting therapeutical efficacy.The second part of the project will provide precious information on the active role of water used for veterinary treatment and on antibacterial drug stability and solubility, by applying real " field condition", we could address the correct use of antibacterials, improving animal, human and environmental health (4).

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EVALUATION OF ANGIOGENESIS IN CANINE PROSTATIC TUMORS BY QUANTITATIVE IMMUNOHISTOCHEMISTRY

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Canine prostatic tumors, differently from those of man, don't recognize a hormone-dependent mechanism (Bell et al.1991). Castration seems to increase the incidence of prostatic tumors in young dogs, by inducing an increase in activation of the angiogenesis process (Padley et al.2002), which is essential for tumor growth (Fox et al.,1996).The aim of this study was to study angiogenesis, by evaluating vascular endothelial growth factor (VEGF) expression, and microvessel density (MVD), in canine hyperplastic and neoplastic prostates.

20 samples of canine prostates (10 hyperplasias, 8 carcinomas, 2 normal) were routinely processed, classified according to WHO criteria (Kennedy et al.,1998) and immunohistochemically labeled with VEGF and Von Willebrand factor antibodies. MVD, vascular parameters (luminal area and perimeter) and VEGF expression were evaluated by the same procedure as that used by Restucci et al. (2002).

MVD and VEGF expression increased gradually proceeding from normal samples, to prostatic hyperplasia and malignant tumors. Vascular parameters appeared larger in hyperplastic samples than in normal ones and decreased in malignant tumors, in which they showed small and often malformed lumens.

The increase in VEGF synthesis and MVD in hyperplastic and especially neoplastic samples confirms the role played by angiogenesis in the malignant progression of canine prostate, as demonstrated in other canine tumors (Restucci et al., 2002; Maiolino et al., 2000). The decrease of vascular parameters is also, in our opinion, linked to the abnormal increase of VEGF synthesis, not balanced by an adequate production of other angiogenetic factors, necessary to form well-structured vessels. Our results allow us to hypothesize that angiogenesis can be one of the main mechanisms of tumor growth in canine prostate.

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	Normal prostates	Hyperplastic Prostates	Neoplastic prostates	P Value
VEGF POSITIVE CELLS	6,1±1,5	19,6±8,4	106,9±32,07	P<0.001
VESSELS NUMBER	49+±113	97,83±53,7	263±35,3	P<0.001
AREA µ/m²	73,4±32,2	129,39±125,2	65,05±4,3	P<0.001
PERIMETER µ/m	36,6±7,4	40,7±12,4	39,7±0,2	P=0.009

CARDIAC PATHOLOGY IN BLUEFIN TUNA (THUNNUS THYNNUS)

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Little is known about cardiac pathology of Bluefin Tuna. Aim of this work is to evaluate gross and histopathological cardiac lesions of regularly fished tuna.

Sixteen hearts of Bluefin tuna (Thunnus thynnus) fished in the Ligurian Sea of Italy were examined. Weight and measures (length of the caudal furca) were recorded, and samples were collected on boat and stored in 4% buffered formalin for gross and histologic investigations, stained with Haematoxylin-Eosin, Periodic Acid Schiff, Toluidine blue and Alcian blue.
Gender was determined by macroscopic observation of the gonads. The age of all animals was estimated by counting the bands of skeletal growth on the first fin rays of the first dorsal fin. Serial sections 1.0 mm thick of the condyle base were obtained, dried for 24 hrs, observed with a stereo microscope, and the number of rings was counted to assign an estimated age.

Eight males and 8 females aged between 3 and 12 years were analyzed. Macroscopically in two cases the hearts showed a fibrinous pericarditis also associated, in a further case, with small, variably shaped, 1-2 mm in diameter nodules, scattered on the surface of the atrium, epicardium and bulb. In the latter case the myocardium revealed a focal, white, dry, well demarcated nodule, with a necrotic core. Histological examination of the pericardium showed in three cases a fibrinous pericarditis associated with diffuse mononuclear cells, in one case a perivascular cuffing, and in another case Ichthyophonus spp. granulomas. The myocardium showed in 10 cases myocarditis, in 4 cases focal fibrosis, in 3 cases Ichthyophonus spp. granulomas, in 2 cases arteritis, in one case a focal replacement of the fibers by adipose tissue, and in one case arteriolar stenosis. Flogosis was present in six cases and in one case Ichthyophonus spp. granulomas were observed in the bulb. Endocardiosis (11 cases), endocarditis (9 cases), thrombi (5 cases), Lambl's excrescences (4 cases), and valvulitis (1 case) were histologically detected in the endocardium.

Heart lesions of Bluefin Tuna are very complex and appear different in prevalence and severity, compared to lesions observed in other fishes catched in the Ligurian Sea. Most of the lesions show an inflammatory or degenerative pattern, with a low prevalence of a parasitic etiology. It is relevant the finding of a few cases of Ichthyophonus spp. infection, already reported as associated with adverse flesh quality and possibile losses, although without any zoonotic implication. Further studies on the etiology of these lesions are needed to better understand their pathogenesis, and to compare these data with cardiac pathology of mammals and humans.

PATHOLOGY OF FREE-LIVING LOGGERHEAD TURTLES (CARETTA CARETTA) EMBRYOS ON THE ISLAND OF LINOSA (ITALY)

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Three endangered species of sea turtles are present in the Mediterranean Sea. In particular, Caretta caretta has been included in the category "critically endangered species".
There is a lack of studies about the earlier development stages such as incubation and hatching, and the purpose of this work is to evaluate the cause of death of not hatched specimens of Caretta caretta from Linosa Island.

Forty-seven Loggerhead Sea Turtles (Caretta caretta) found dead at the opening of the nests on Linosa Island beach (Italy) in summer 2006 were examined. Biometrical measures and depth of the eggs were recorded. Samples were submitted to histological examination and stained with Haematoxylin-Eosin, Grocott, Periodic Acid Schiff, Von Kossa and Movat pentachrome. Immunohistochemistry for Herpesvirus was performed. July and August temperatures of the Linosa Island from 2004 to 2008 were registered. Statistical analysis were performed using Fisher's exact test, chi-squared and Kruskal-Wallis test.

Biometrical measures showed that 17 (41%) animals were hatchlings and all the other animals were in the last third of development. In 3 (6%) cases a focal non-suppurative infiltration of the heart was observed. An increasing amount of melanomacrophages (9 cases; 19%), haemorrages (3 cases; 6%) and vacuolar degeneration (47 cases; 100%) were present in the liver. Edema was observed in the lung (8 cases; 17%) and 26 (59%) animals revealed glomerular and tubular calcium carbonate calculosis involving over 50% of the renal parenchyma in 19 of 26 animals (70%). Immunohistochemistry was negative for Herpesvirus.
Statistical analysis revealed an association between nest and renal calculosis and between differences of the average temperature of July and August and the other considered years.

The most significant lesions were present in the liver and kidneys. Vacuolar degeneration of the liver associated with increased melanomacrophages is indicative of a chronic inflammatory process probably due to toxicosis. The presence of calcium oxalate crystals in the kidney is believed to be linked to egg dehydration due to increased environmental average temperatures. Therefore it is supposed that the cause of the hatching failure was the dehydration of eggs and that the hepatic lesions were due to a chronic degenerative process associated with the vertical transmission of toxic substances.

CARDIAC PATHOLOGY OF THE SWORDFISH (XIPHIAS GLADIUS).

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The heart of marine teleosts can be affected by several infectious and parasitic diseases. The aim of this study is to evaluate the alterations of swordfish hearts compared to lesions detected in mammals and human.

Twelve hearts of swordfish (Xiphias gladius) fished in the Ligurian Sea of Italy in 2012 were submitted to gross and histologic investigations, stained with Haematoxylin-Eosin, Periodic Acid Schiff, Toluidin blu and Alcian blu.

Epicardium: 11 out of 12 hearts showed an inflammatory reaction of the pericardial sac, with diffuse fibrinous pericarditis and involvement of the bulb. Discrete blood collections and yellow, dry, miliary foci characterized by a necrotic cut surface between the epicardium and pericardium were observed. In one case, a gray-whitish discolouration involving the bulbo-ventricular valves was present.
Histopathologically a considerable fibrous thickening of the pericardium consisting in connective fibers infiltrated by lymphocytes and rare neutrophils, and neoformation of capillaries within the pericardial connective tissue were evident. Small necrotic granulomas arranged in chains along the epicardium due to Ichthyophonus spp. were also detected in 2 cases. Granulomas were surrounded by a fibrous connective tissue capsule, and by lymphocytes and rare neutrophils.
Myocardium: at gross examination the myocardium appeared normal, while histopathological evaluation showed lymphocytic perivascular cuffs in the compact layer (6 cases), fibrotic foci replacing the myocardial fibers (4 cases), and a focal replacement of fibers by adipose tissue (1 case). Granulomas due to Ichthyophonus spp. were detected in 4 cases.
Endocardium: the following alterations were detected in the endocardium: valvular fibrosis of atrio-ventricular and bulbo-ventricular valves (7 cases), Lambl's excrescences (7 cases), endocardiosis (6 cases), Ichthyophonus spp. granulomas (2 cases), and valvulitis (1 case).

Swordfish cardiac pathology shows interesting findings which should be considered for comparative pathology, also in consideration of possible environmental factors. To the authors' best knowledge Lambl's excrescences and endocardiosis have never been previously described in swordfish.

IDENTIFICATION OF CETACEAN STRANDED ON THE SICILIAN COAST BY DNA BARCODING

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Dolphins are widely distributed across the warm-temperate to tropical waters of the world. About this, Stenella is a cosmopolitan cetacean and the most abundant dolphin in the Mediterranean sea. Genetic markers have been used in many cetacean species to describe genetic diversity and to identify genetic differences in the populations. Stranding of dolphins along the Italian coast have been reported in the last period. It has been shown as mtDNA genes sequencing analysis could be useful to identify the cetacean when the carcasses do not allow to recognize the species. In the present work, we report species identification regarding cetacean stranded on the Sicilian coast from January to date. The aim of the study was to evaluate the cetacean species involved and to understand if there was a species-specificity stranding, as well as to make a valuable contribution to the molecular genetic investigations of the Mediterranean population of dolphins.

Samples were collected from 20 cetacean carcasses founded in various part of the Sicilian coast. Genomic DNA was extracted and a mtDNA region was amplified and sequenced based on cytochrome b target. Data analysis was performed by using appropriate software as tool to demonstrates the phylogenetic linkage trough the investigated animals.

Results showed that samples belong mainly to the striped dolphin species, Stenella coeruleoalba (n=16). The remaining species belong to the bottlenose Tursiops truncatus (n=2), Balaenoptera physalus (n=1) and Delphinus delphis (n=1).

In this study, the authors identified by DNA barcoding 20 stranded cetacean. Today it is not yet clear the cause of strandings and why only affect cetaceans. Probably a morbillivirus infection could be the responsible as demonstrate by the findings of the parasitized carcasses. In conclusion, mtDNA region with a high degree consensus, such as Cyt-b genes, have proved highly informative power for species identification and genetic population studies. Furthermore it is suitable for studies of genetic variability, phylogeography and forensics

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LOCAL DELIVERY OF EMBRYONIC STEM-LIKE CELLS INTO OSTEOCHONDRAL DEFECTS IN SHEEP FEMORAL CONDYLES: HISTOPATHOLOGICAL EVALUATIONS AND FISH DETECTION.

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Articular cartilage is unable to regenerate itself because of its avascularity and slow metabolic turnover. Width and depth of degenerative processes, frequently occurring in the regenerated tissue (1) affect their fate: defects 3 mm wide re-fill with hyaline cartilage, while wider defects are replaced by fibrocartilage (2); moreover, chondral defects do not heal spontaneously 1, 2, 6, while osteochondral defects, allowing the access to the mesenchymal stem cells (MSCs) from the bone marrow can completely heal. Embryonic stem cells (ESCs), being able to differentiate into tissues from all 3 germ layers (3) represent the most valid cell type. This study aimed to histologically evaluate if the local delivery of ES-like cells into osteochondral defects in the medial femoral condyles of sheep may enhance the articular cartilage regeneration.

ES-like cells embedded in fibrin glue were engrafted into full-thickness osteochondral defects in the medial condyles (ES) of the left femur in 22 ewes. An identical defect was created in the controlateral stifle joint and left untreated as a control (empty defect: ED). The ewes were divided into 5 groups and euthanized at 1, 2, 6, 12 and 24 months from surgery. The evaluation of regenerated tissue was performed by macroscopic, histological, immunohistochemical (collagen type II) and fluorescent in situ hybridization (FISH) assays.

No significant differences were found between treated and control sites in the macroscopic assessments at any time point. ES treatment had significantly better histologic results in respect to ED throughout all considered periods. The FISH confirmed that the regenerative tissue originated from the ES-like cells.

Histological results showed a significant better overall healing in ES throughout all time periods, as well as a better performance in certain specific categories. In particular, in the later periods ES showed more normal histological cartilage architecture as compared to ED, with one sample at 24 months post-surgery showing regenerated tissue comparable to normal hyaline cartilage, and a significant higher production of proteoglycans and collagen type II, which are fundamental for the stiffness of the matrix

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MICRORNA EXPRESSION IN HUMAN AND CANINE OSTEOSARCOMA: BIOMOLECULAR AND COMPARATIVE STUDIES

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MicroRNA expression in human and canine osteosarcoma: biomolecular and comparative studies.
Leonardi L., Benassi M.S., Di Guardo G., Quattrini I., Roperto F., Picci P., Del Piero F., Pazzaglia L.

Osteosarcoma is the most common primary malignant bone tumour in dogs and young-adult humans. MicroRNAs are short non-coding RNA molecules involved in post-transcriptional gene expression that play an important role in tumour cell proliferation. Evidence suggests that miRNAs may act as tumour suppressors or as oncogenes that control growth and apoptosis and recently distinct miRNA expression signatures have been proposed as diagnostic and prognostic markers for various types of human cancer including sarcomas. The aberrant expression of miRNA 196a is a frequent event in human neoplasia (colorectal carcinoma, gastrointestinal stromal tumour, breast carcinoma, non-small cell lung carcinoma, adenocarcinoma) and although a more frequent overexpression was found, it could also play a tumour suppressor role showing a reduced expression in melanoma cells when compared to healthy control patients.

The study was carried out on 32 frozen high grade OS samaples from patients referred to the Rizzoli Orthopaedic Institute. Twenty high grade OS frozen samples from large size dogs were obtained from the University of Perugia. Twelve were male and 8 female with a mean age of 8 years. In all specimens the percentage of tumour cells estimated after haematoxylin-eosin staining of tissue sections contiguous to those used for the study was equal or more than 90%.

A down-regulation of miR-196a was seen in both human and canine tumours when compared with species-related normal bone, highlighting the role of miR-196a in osteosarcoma development of both species. When we analyzed the impact of ectopic miR-196a expression in order to compare the cell response in different species, a slowing down of migration associated or not with changing in proliferation and apoptosis was seen.
Based on clinical behavior, as well as on histological and molecular features, canine and human OS show a significant homology, making the dog a valuable comparative pathology model for the study of osteosarcoma in human. Similarities include metaphyseal regions of long bones localisation, histological features, the high incidence of high-grade tumours and some genetic aberrations such as p53, CDKN2A, PTEN and RB1.

In conclusion, the results presented herein highlight the role of miR-196a in both human and canine OS development and progression, thereby reinforcing the importance of the dog as a potential model for the study of OS in human and the need for further comparative analyses and translational studies. Although this study may reinforce the importance of the dog as a potential model for further comparative studies, cell response to miR-196 overexpression seems to be more strictly related to cell type/species and cell functional characteristics.

UNUSUAL ANATOMO HISTOPATHOLOGICAL FINDINGS OF TUBERCULOSIS INNEBRODI BLACK PIGS.

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The aim of the present study is to report the most significative pathological findinds associated with tuberculosis in Nebrodi Black Pigs of Sicily detected in 2013. Swine is receptive to different mycobacteria: M bovis, M.avium, M.microti. The epidemiological role of the domestic Black pigs in the maintenance of bovine tuberculosis is until now unclear. Usually the infection is oral and the primary complex affected head and/or mesenteric lymph nodes. The respiratory tract is rarely involved.

In the first half of 2013, 299 Nebrodi Black pig carcasses were submitted to a complete inspection during slaughter. Macroscopical and histopathological investigations were carried out.

In 20 (6,6%) of the carcasses localized granulomatous lesions were reported involving almost exclusively lymph nodes. In two subject, a young male and a sow, macroscopic generalized lesions involving tonsils, udders and coxo-femoral joint were detected. Particularly in the tonsils small translucent gray nodules, detectable both in surface and in section, were observed. In three udders multiple granulomatous lesions of different sizes, often involving the lumen of the papillary ducts were identified. A voluminous neoformation (10 cm) was observed in the right coxo-femoral joint. Bone metaplasia of the joint capsule with areas of necrosis and inflammation was present. The X-ray examination revealed bone remodeling of the trochanter and ischial bones with osteolysis. Histologically lesions were classified as granulomatous or atypical. Granulomatous lesions appeared mostly as initial granulomas composed of mononuclear inflammatory cells without a necrotic centre or aggregate of polymorphonuclear cells or as classical fibronecrotic calcified granulomas. Atypical lesions were recorded mostly as haemorrhagic foci or neutrophil aggregates present in many cases positive for M. bovis.

Granulomatous generalized lesions in the udder allow to hypothesize the transmission through the milk. In author's opinion this is a rare report of tuberculous lesions in swine tonsils caused by M. bovis.

V. Di Marco.et al.: Epidemiological Significance of the Domestic Black Pig (Sus scrofa) in Maintenance of Bovine Tuberculosis in Sicily.J. Clin. Microbiol. 2012, 50(4):1209-1218

MUSCOLOSKELETAL MALFORMATIONS IN SHEEP IN SICILY: PERSONAL EXPERIENCES OF THE AUTHORS

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The purpose of this study is to describe the musculoskeletal malformations observed in Sicily during the diagnostic activity of the authors. Congenital malformations affect all species of domestic animals. Their causes range from genetic to environmental possibilities or a combination of both. Moreover other factors like breed, geographical area, year, sex, parental age, nutrition, could influence the incidence of these alterations. The defects may affect a single structure or function or involve several body system and functions. The results are economic losses, decrease of the maternal productivity, increase of the perinatal mortality and reduction of the value of the defective animals.

From 2007 to 2013 100.000 lambs in various flocks of Sicily belonging to different typical Sicilian breeds like Pinzirita, Comisana, Valle del Belice and their crosses were observed by the authors during the diagnostic activity

The most important musculoskeletal defects observed were the following: increased convexity of the cranial vault (30), cleft palate (palatoschisis) (2), deviation of the nasal bones (1), inferior brachygnatism (100), agnathia (10), deviations of the spine (45), schistosoma reflexus (3), micromely (5) often associated wih syndactyly; perosomus elumbus (1), bifid spines (2), arthrogryposis (20), ligament laxity (20) especially of the fetlock and hip joints, supernumerary ribs (2) and generalized muscle hypertrophy (1). Most malformations were simultaneously present in the same animal.

Border disease and Blue tongue virus infections were correlated to the increased convexity of cranial vault and to stiff neck and arthrogryposis respectively. The authors recognize as possible causes of other malformations insufficient space in the uterine lumen, or teratogenic plants. Moreover it could be suggested but not proven that many observed malformations are inherited. In author's opinion the musculoskeletal malformations of sheep are underestimated because often they are not reported.

M.L. Sonfada, M.N. Sivachelvan, 2Y. Haruna, I.M. Wiam and A. Yahaya. Incidence of Congenital Malformations in Ruminants in the North Eastern Region of Nigeria. Int J Anim Vet Adv 2(1): 1-4, 2010.

BOVINE EOSINOPHILIC MYOSITIS: ROLE OF SARCOCYSTIS SPP.

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Bovine Eosinophilic Myositis (BEM) is an inflammatory condition characterized by multifocal grey-green lesions in striated skeletal muscle, esophagus and heart. This pathology is not detectable in the living animal, because affected animals appear clinically normal, but it results in economic losses due to carcass condemnation at slaughter. Sarcocystis spp. have been suggested to play a role in this myositis even if the prevalence of BEM is very low, while the prevalence of Sarcocysts spp. in cattle is extremely high. The aim of the present study was to describe the histological features associated with BEM and to present the preliminary investigations to determine if BEM is associated with Sarcocystis spp. infection.

From 2008 to June 2013 samples from 22 bovine muscle with macroscopical lesions of BEM were examined. From each muscle after macroscopical evaluation samples were fixed in 10% buffered formalin and paraffin embedded to perform histological sections routinely processed and stained with haematoxilin and eosin. Samples from each muscle were also -80°C frozen to perform biomolecular investigations with a Multiplex PCR assay for the identification of Sarcocystis spp. in cattle.

In all muscle the diagnosis of BEM was confirmed by histology. The inflammatory infiltrates consisted mainly of eosinophilic granulocytes, but lymphocytes, plasma cells and macrophages were frequently observed. In 13 cases (59.09%) granulomatous lesions with multifocal calcified necrotic fibers, mixed inflammatory cells mainly consistent with eosinophils, epithelioid cells and multinucleated giant cell were detected. Intralesional sarcocysts were detected in 2 cases (9%). All intralesional sarcocysts were damaged and located in the central necrotic core of granulomatous lesions. Extralesional intracellular sarcocysts located in intact muscle fibers not surrounded by inflammation were detected in 5 cases (22.7%). There was no evidence of parasite infection in n 15 samples (68.18%). Granulomas may have resulted from Sarcocystis spp. infection that served as chronic inflammatory stimuli, even if no parasites were detected.
Preliminary biomolecular investigations revealed, in the majority of cases, the intralesional presence of Sarcocystis hominis DNA.

Due to the zoonotic potential of Sarcocystis hominis, although the direct association sarcocystosis/BEM in most of cases was not ascertained, the authors suggest to increase the biomolecular investigations on BEM cases and to activate procedure to prevent the transmission of Sarcocystis spp. oocysts to cattle and consequently to human people.

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EPITHELIOTROPIC VIRAL INFECTIONS IN DEER FROM THE CENTRAL ALPS

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Starting from winter of 2008, several cases of skin infections characterized by proliferative lesions, have been detected in red deer (Cervus elaphus), chamois (Rupicapra rupicapra) and ibex (Capra ibex) from the central Alps. The purpose of this work was to characterize the genome of the epitheliotropic viruses isolated from skin lesions of these animals.
The families Papillomaviridae and Poxviridae include numerous DNA viruses affecting several mammal species. The genus Parapoxvirus, included viruses wich causes pustular lesions to small and large ruminants[2]. The family Papillomaviridae comprises the genus Deltapapillomavirus, of which some species can infect wild and domestic ruminants[1,4]. They are responsible for the formation of fibropapillomas[3].

Firstly, all the samples were submitted to EM analyses and subsequently PCR was carried out on PPV positive samples with the aim of amplifying the B2L and the VEGF genes. The DNA purified from sample 1127, which has showed the presence of papillomavirus particles by EM, was then analysed by PCR to amply a fragment of the gene coding for either the L1 protein, and the protein E5. All the PCR products were purified and sequenced, and the nucleotide and amino acid sequences were aligned and compared with the reference strains, finally, phylogenetic analyses were carried out.

In all the PPV positive samples by EM were also PCR amplified, and the sample 1127 confirm to be infected by papillomavirus either by EM and PCR. The analysis of the nucleotide sequences of parapoxvirus from deer showed, for the first time, the presence of PVNZ in wild ruminants outside New Zealand. Phylogenetic analysis suggested that the PVNZ might be the product of the recombination of two distinct species of parapoxvirus (PCPV and BPSV); the analysis of the nucleotide sequences of parapoxvirus isolated from chamois and ibex instead have confirmed that these animals are susceptible to infection by orf virus. To finish, the phylogenetic analyses performed on the papillomavirus L1 sequence of 1127 strain, showed a highest similarity with the Capreolus caprelus papillomavirus CcaPV1, and a concomitant co-infection with BPV-1.

Our results demonstrate that deer confirm to be permissive hosts for epitheliotropic DNA viruses, and that might behave as possible mixing vessel, leading the possibility for interspecific recombination for different viral species and potential for recombination events.

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PSEUDOTUBERCULOSIS IN ALPACAS (LAMA PACOS): ANATOMOPATHOLOGICAL AND BACTERIOLOGICAL FINDINGS

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Pseudotuberculosis is a transmissible infectious disease caused by Corynebacterium pseudotuberculosis, that is mainly reported in european small ruminants, but can affect horses, dairy cattle and new world camelids, and occasionally buffaloes, camels, deers, pigs, primates and ducks (Baird et al, 2007). In new world domestic camelids as Alpaca (Lama pacos) and Llama (Lama lama) C.pseudotuberculosis infection is reported in association to suppurative lymphadenitis, mainly in renal lymph nodes in adult alpacas and in superficial lymph nodes in younger alpacas (Braga, 2006). Other affected organs are mammary gland, and rarely liver and lungs. The aim of the work is to report pathological and bacteriological findings in a large series of 18 spontaneous cases of C.psudotuberculosis infections in a farm breed in central Italy.

The cases have been observed between 2004 and 2012 and involved young and adult animals, both males and females.

The main pathological finding was represented by skin abscesses associated with regional lymph node involvement. In 9 cases a multifocal dissemination to lungs in the form of scattered nodules raging from 1 to 10 mm of diameter. Liver involvement was micronodular in 3 cases and severe multinodular to coalescing in 9 cases. In 3 cases mesenteric lymph nodes had multiple scattered pinpoint small microabscesses. Other lesions occasionally observed were located to mammary glands, kidney, costal bones and heart . Histologically the lesions were dominated by infiltration of macrophages with epithelioid appearance and degenerated neutrophils that border a large amount of eosinophilic amorphous material with necrotic debris. Swabs form abscesses have been coltured on Blood Agar with 5% sheep blood in aerobic conditions at 37°; after 48h C. pseudoyuberculosis colonies were small, whitish and dry, composed by pleomorphic Gram povitive bacteria. Bacterial DNA have been extracted by commercial QIAGEN DNeasy Blood and Tissue Kit according to manifacturer's instructions, and quantified by Nanodrop R. A PCR for ribosomial subunit 16s was run and the amplified identified as a 815bp band on Agarose gel 1,5%. The amplified was then sequenced and found to be analogous with ovine and caprine isolates.

In summary the pathological features of C.pseudotuberculosis infections in alpacas are more heterogeneous than in sheep or goats, with lesser involvement of skin and lymph nodes and a frequent generalization to internal organs, mostly lungs and liver; the massive involvement of liver together with the presence of lesions in mesenteric lymph nodes could suggest an alimentary route of infection, in addition to trans-cutaneous one. The genetic homology between isolates in alpacas and sheep could suggest that the susceptibility of alpacas to the disease is related to species specific factors rather than to differences in virulence among bacteria.

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Braga et al., Vet Rec 2006 159 : 23-24

SMALL RUMINANT MASTITIS: EPIDEMIOLOGICAL SURVEY AND PATHOLOGICAL INVESTIGATIONS

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The purpose of this study is to describe the epidemiology and pathology of mastitis in dairy sheep farms of Sardinia and Sicily, in order to underline the relevance and impact of this disease on veterinary public health. The most significant cases collected during the activity survey are also reported. Mastitis represents the main disease in dairy small ruminants herds, affecting the animal welfare and causing considerable economic losses. Udder infection in dairy small ruminants is considered to have major negative effects on both yield and quality of milk, and leads to greater economic losses than those reported for dairy cattle. Staphylococci are the main aetiological agents of small ruminant mastitis and particularly Staphylococcus aureus and coagulase negative staphylococci (CNS) species. Currently, CNS are the most commonly isolated mastitis-causing agents in dairy ruminants in many countries and they are considered emerging mastitis pathogens. In the South of Italy, especially in Sardinia and Sicily, small ruminants farming is one of the most important resources for the regional economy. Trappetti et al. (2007), on ovine flocks with mastitis of Sardinia, indicated CNS as the major aetiological agents.

A preliminary epidemiological study from 2004 to 2011 based on bacteriological analysis of mastitic milk samples was perfomed on 1046 flocks. Pathological investigations including macroscopical and histological evaluations were made on selected mastitic mammary gland tissues.

The survey performed in Sardinia region confirms the most important role of CNS in causing intramammary infection with a frequency of isolation (FI) of 48.9 ± 2.7% (S. chromogenes and S. epidermidis particularly), followed by S. aureus (FI 31.1 ± 1.9%), Streptococcus uberis (FI 15.9 ± 0.8%) and Pseudomonas aeruginosa (FI 13.8 ± 0.8%).
In Sicily Di Marco et al. (1996) reported S. aureus and Mycoplasma agalactiae as responsible of most clinical mastitis, followed by CNS. Recently Scatassa et al. (2012), in 5 flocks M. agalactiae-free (14072 milk samples), confirmed staphylococci as the major agents of ovine mastitis (prevalence rates of 80% for CNS; 10.9% for S. aureus; 0.69% for S. intermedius and 0.16% for S. hyicus) followed by Streptococcus spp. (5.64%) and Mannhemia haemolytica (0.63%).
Macroscopical and histological evaluations revealed in most cases moderate to severe suppurative inflammation, necrosis and fibrosis.

The present study confirms Staphylococci as the main aetiological agents of small ruminant mastitis. Concern is the steady increase of environmental mastitis over the last years particularly difficult to eradicate.

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Scatassa et al., 2012. Cellule somatiche ed isolamento di agenti mastidogeni …., XX Congresso Nazionale SIPAOC, Siracusa, 26/29-09-2012. P: /
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EXPERIMENTAL INFECTION WITH STREPTOCOCCUS UBERIS IN SARDA SHEEP: HISTOPATHOLOCICAL EXAMINATION AND INFLAMMATION CHARACTERIZATION

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Streptococcus uberis (Su) is the main environmental pathogen that causes mastitis in dairy animals, the infection produces predominantly subclinical mastitis. The topic has been recently studied by experimental infection in goats (1). Knowledge of mastitis caused by Su in sheep are poor; we have reproduced experimentally this type of mastitis in order to understand the pathogenic mechanism, the characteristics of the inflammation and to investigate the innate immune response mediators released in milk by mammary epithelial cells (MECs).

The experimental infection was carried out in 5 Sarda sheep, first-time pregnancy, the second month of lactation and free of mastitis; evaluated through clinical examination and microbiological culture of milk, the serum negativity for Su was also evaluated. The inoculum was carried out using 2 x 107 cfu of Su via the teat canal on the left half-udder of 4 sheep, in the animal control was inoculated sterile PBS.
Samples of blood and milk was collected daily and, 6 days after, we proceeded to the euthanasia and autopsy of the animals, with particular attention of the breasts and lymph nodes. Samples of organ was taken from different districts, fixed in 4% formalin for histological examination, and frozen at -80°C .

The establishment of infection was confirmed both clinically and by molecular methods, including PCR and FISH on mammary tissues. Proteomic investigation of the milk enabled to detect an enrichment in proteins involved in inflammation, chemotaxis of immune cells, and antimicrobial defense in infected animals, suggesting the consistent involvement of mammary epithelial cells in the innate immune response to pathogens (2). All infected animals showed an increased volume of lymph nodes of the inoculated half-udder. Histopathological grading score of mammary tissues highlighted a clear difference between infected and uninfected udder halves. The control animal and uninfected halves showed no signs of inflammation and the epithelium was intact.

Our results support an active role of MECs in the innate immune response of the mammary gland, and provide new potential for the development of novel and more sensitive tools for monitoring mastitis in dairy animals. Further studies are needed to better understand the pathogenesis, the response of the immune system and characterize the strains of Su inducing mastitis.

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EFFECTS ON TESTIS OF HORMONAL IMPLANTS IN BEEF CATTLE

Pregel P.*[1], Cannizzo F.T.[1], Divari S.[1], Ramella Pralungo C.[1], Ferrari E.[1], Starvaggi Cucuzza L.[1], Salamida S.[2], Manuali E.[2], Biolatti B.[1]

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Growth promoters (GPs) administration in livestock is a major health problem for veterinary public health, with serious implications for consumers. Sex steroids and corticosteroids are the substances most frequently fraudulently administered, especially in beef cattle. The use of subcutaneous sex hormones implants, legal in USA, is absolutely banned in Europe. These substances have been associated to corticosteroids during the finishing period in cattle. Subcutaneous implants, containing ester forms of GPs, consist of prolonged-release formulations. Testis is a target organ of GPs, therefore the aim of this study was to evaluate the effects of subcutaneous implants on testis.

Thirty-two Friesian beef cattle were experimentally treated with subcutaneous implants containing sex hormones (8 animals/group), as follows: group R received subcutaneous implantation of Revalor-200 (trenbolone acetate and estradiol), group RD was administered of 0.7 mg/animal/os per die of Desashock per 40 days, in addition to Revalor implantation, and group F received subcutaneous implantation of Finaplix-H (trenbolone alone). Group C represented the control group. The animals were slaughtered 90 days after the implantation and 6 days after last Desashock administration. Morphological and molecular studies were carried out on testis samples, using optical and electron microscopy, TUNEL, immunohistochemistry, and qPCR. The main objectives were to evaluate the influence of treatments on spermatogenesis, cell proliferation and apoptosis in testis. Moreover, Regucalcin (RGN) expression and oxytocin pathway were investigated.

Morphological analyses showed slight alterations in the testicular tissues of treated animals, with a reduced presence of spermatozoa in epididymal ducti, but, in spite of a significant reduction of testis relative weight in treated groups, morphometrical analyses did not reveal alterations in the architecture of the tissues or in the relative quantity of each cell type. This finding was also confirmed by qPCR analysis of genes differentially expressed during spermatogenesis (COX6B2, PRM1, PRM2, TNP1, HSP2 and AMH) and apoptosis (BAX, Bcl2, GR). TUNEL and Ki-67 antigen expression were not significantly different in the groups. Oxytocin precursor and oxytocinase genes expression did not vary significantly between the groups, whereas Oxytocin Receptor (OXTR) gene was significantly down-regulated both in RD ($p<0.05$) and R ($p<0.01$) groups and RGN in all the examined groups ($p<0.01$).

In comparison with results obtained in a previous experiment in animals treated with estradiol and dexamethasone, the morphological and morphometrical effects of the implants containing association of low doses of different GPs were difficult to detect, even with the help of sensitive techniques. The results about OXTR gene and RGN suggest to further investigate the role of these markers in testis for the detection of illicit treatments, even at low doses or with cocktails.

MOLECULAR APPROACHES FOR THE IDENTIFICATION OF GENETIC SUBTYPES OF STAPHYLOCOCCUS AUREUS ISOLATED FROM MAMMARY INFECTION

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S. aureus is one of the most important causative agents of bovine mastitis and the clinical outcome can vary in relation to the strains involved in the mammary infection. The aim of this study, funded by Lombardy Region, was to develop a diagnostic protocol for the identification of genetic subtypes of S. aureus responsible for bovine mastitis, with particular virulence and diffusiveness.

S. aureus strains associated with intra-mammary infections were isolated from 8 "control" (< 4% of infected cows) and 8 "case" (> 28% of infected cows) herds for a total of 2015 cows included in the study. Milk samples were cultured with standard methods and the DNA was extracted from S. aureus strains using a protocol described in literature (Cremonesi et al., 2006). Twenty-four and 626 strains isolated from single quarters of "control" and "case" herds respectively, were analysed by both RS-PCR (Fournier et al., 2008) and multiplex PCR. The first method is based on the amplification of the 16S-23S rRNA intergenic spacer region, the multiplex PCR targets the genes encoding for enterotoxins and other virulence genes such as leukocidins and leukotoxins. A subset of 55 strains representative of “control” and “case” isolates was also analysed by ribotyping (automatic Riboprinter), by Identibac array tube system and by protein profiling using MALDI-TOF MS.

The RS-PCR analysis of the 650 strains revealed 10 different profiles. In 5 out of 8 “case” herds this method identified the genotype GTB and GTB-variant indicated in literature (Graber et al., 2009) as characteristic of highly diffusive and pathogenic strains, while GTK, GTR_VI and a new genotype named GTbM circulated in the remaining 3 herds. Seven out of the 8 "control" herds, revealed only one S. aureus profile with a predominance of the GTS genotype.
Except for 4 out of the 55 representative strains analysed, ribotyping gave a response similar to RS-PCR. Moreover, MALDI-TOF and Identibac analyses proved to be able to integrate the molecular profiling of S. aureus isolated in field.

Our results confirmed that RS-PCR could be used as a rapid test for molecular typing of S. aureus strains isolated from bovine mastitis and could identify genetic subtypes with particular virulence. Ribotyping, MALDI-TOF and Identibac analyses showed their capability for molecular strains characterization, integrating RS-PCR data. Multivariate analysis by combining data from different techniques could be used to discover a simple panel of markers associated to S. aureus virulence and diffusiveness.

Cremonesi P., et al. (2006). J. Dairy Sci., 89:163–169; Fournier C., et al. (2008). Res. Vet. Sci., 85: 439-448; Graber HU., et al. (2009). J. Dairy Sci., 92(4) 1442-1451

IN VITRO BIOFILM FORMATION AND PATHOGENIC PROFILE OF ESCHERICHIA COLI ISOLATED FROM MEAT PRODUCTS

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Meat from ruminants is one of the main sources of VTEC human transmission (Karmali, 2010). E. coli can persist in the farm environment, soil, water, sediment and animal carcasses (Krzyminska, 2010). Several studies demonstrated that E. coli, and other genera of the Enterobacteriaceae, are able to form biofilms on a variety of surfaces (Rivas, 2007). The aim of the present study was to evaluate the in vitro biofilm forming ability (BFA) and the pathogenic profile of 95 presumptive VTEC, isolated from fresh meat and meat products.

All the samples were submitted to a direct PCR screening method, to detect the stx genes. Positive enrichment broths were then submitted to immunoseparation, by using the Dynabeads anti-E. coli O157, O26, O103, O111 and O145 serogroups, and isolation on CT-SMAC, CT-RMAC and EHLY agar. Virulence factors were determined for all the isolates by multiplex PCR (Mazzette, 2012). E. coli O157 latex agglutination test and the BFA, on microtiter plates, were also performed (Rivas, 2007).

The 17.5 % of the isolates resulted E. coli, but none belonged to VTEC and to O157 serogroup. The 82.5 % belonged to other Enterobacteriaceae (Enterobacter cloacae, Hafnia alvei). The 82.4% of the E. coli strains were positive for the BFA. In particular 64.7% resulted weak-, and 11.8% moderate-producers. No strong-producers were detected. None of the E. coli showed virulence genes, while two strains among the other species (Enterobacter cloacae) were eae-positive.

The results confirm the widespread dissemination of E. coli biofilm-forming (Hurrell, 2009). The low specificity of VTEC isolation methods is also confirmed, and improve the concentration techniques is needed. It is also important to reduce the impact of different factors (thermal stress, PCR inhibitor substances, inhomogeneous microorganism distribution within the samples) to the E. coli vitality, taking into account the higher resistance and competition ability of Enterobacteriaceae. For some species (mainly Citrobacter, Enterobacter, Klebsiella, and Proteus spp.) the potential pathogenicity and a BFA is frequently described as a primary cause of nosocomial infections (Zogaj, 2003).

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Rivas L et al 2007 J Microbiol Met, 69: 44-51.6)Zogaj X et al 2003 Inf Imm, 71, 4151–4158.

RELIGIOUS SLAUGHTER: DATA FROM SURVEYS AND ANIMAL WELFARE ISSUES

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The research aims at identifying the procedures for current methods of religious slaughter in Italy. The observed parameters are discussed in the light of the risk to animal welfare. This research is the first, systematic attempt to analyze the methods of religious slaughter and to discuss the implications for animal welfare.

The list of abattoirs authorized to perform religious slaughter in Italy was requested from the Italian Ministry of Health. Visits to the Italian abattoirs were carried out. All the animals were slaughtered without stunning, in compliance with the derogation permitted by Italian legislation. A reliable, reproducible protocol was developed by means of a video-audio recording of each animal during slaughter.

Conventional slaughter is performed with prior stunning; kosher slaughter is practiced without stunning. Halal slaughter is performed for most of the animals without stunning. Halal slaughter with prior stunning is accepted for 5.9% of small ruminants. Conventional slaughter at the abattoirs interviewed is practiced for 95.3% of cattle, 90.37% of small ruminants and 98.69% of poultry. Halal slaughter is performed for 4.27% of cattle, 5.47% of small ruminants and 1.31% of poultry. Kosher slaughter is practiced for 0.43% of cattle, 4.16% of small ruminants and almost 0% of poultry. For halal slaughter, 100% of cattle, 100% of poultry and 94.1% of small ruminants are slaughtered without stunning, whereas 5.9% of small ruminants are halal slaughtered with prior, head-only, electrical stunning.

The legislation in all EU member states assumes that an animal will suffer less if made unconscious prior to slaughtering. We can conclude that observations and research will strike a balance between religious practices and the scientific minimization of animal suffering. In particular, it is appropriate to specify the notion of animal integrity in every religion and to distinguish it from mere animal vigilance. In fact, based on previous experiences in other European regions, it may be possible to identify techniques that limit the state of animal vigilance without causing any injury that may impair its integrity.

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ISOLATION AND MOLECULAR TYPING OF VEROCYTOTOXIN-PRODUCING ESCHERICHIA COLI (VTEC) IN CALVES OF DAIRY HERDS

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Verocytotoxin (VT)-producing Escherichia coli (VTEC) are causative agents of diarrhoea and haemorrhagic colitis, which can result in neurologic disorders and haemolytic uraemic syndrome (HUS). Human infection may be acquired through the consumption of contaminated water or food, by transmission from person to person or through direct contact with animals. VTEC serogroups mainly associated with human infections are O157, O26, O111, O103, O145 [1]. In 2011 an outbreak of foodborne illness caused by VTEC of serogroup O104 was reported. The strain carried the gene for VT2 but lacked gene for intimin (eae) [2]. The main reservoir of O157 VTEC are cattle, but also non-O157 VTEC have been recovered from bovine faeces [3]. The aims of our study were to assess the prevalence of VTEC in young calves of dairy herds and to determine circulating serogroups.

191 faecal samples from calves (1-36 days of age) were collected between 2010-2013 in 14 herds (8-35 animals per farms). 11 herds without any information about the presence of VTEC were randomly sampled; in the remaining 3 farms, sampling followed the isolation of VTEC during the diagnostic activities (targeted sampling). Faeces were enriched in 0.1% peptone water (PW) and directly plated onto MacConkey agar. DNA was extracted from PW and from colonies with typical morphology. The presence of eae and VT genes (stx1, stx2) was demonstrated with a multiplex PCR. Detection of the serogroups O157, O26, O111, O103, O145 was performed by Real-Time PCR on samples positive for eae and at least one VT; O104 was investigated on samples only positive for VT2. When possible, positive calves were re-examined 2-3 weeks later.

VTEC were found in 6 out of 11 herds randomly sampled and in all three farms where the sampling was targeted, with rate of isolation ranging from 10 to 60%. Overall, VTEC were detected in 37 out of 191 samples: 30 were positive for eae-VT1, 5 for eae-VT1-VT2 and 2 for eae-VT2. Serogroup was determined for 31 VTEC isolated in pure culture: 10 were O111, one O26, while the remaining didn't belong to the serogroups investigated (OND). Moreover, 4 samples were only positive for VT2, but didn't belong to O104. 8 out of 21 calves re-examined some weeks later resulted positive again. 5 of these calves, coming from the same herd, shed VTEC for 4 subsequent samplings, but the isolates belonged to at least 2 different serogroups (026, OND).

Our results showed that young calves are commonly VTEC shedders and in their faeces can be also recovered VTEC serogroups reported in HUS cases. Our data confirm that calves and their environment could play an important role in the transmission of human infection. Further molecular analysis should be performed in order to compare calf strains to VTEC isolated from HUS cases in the same area of study.

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PREVALENCE OF STAPHYLOCOCCUS AUREUS AND MRSA IN BULK TANK MILK OF LOMBARDY DAIRY HERDS

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S. aureus is the most important causative agent of subclinical mastitis in cattle. In the affected farms the clinical outcome and the consequent economic losses can vary in relation to the strains involved in the mammary infection. Methicillin-resistant strains (MRSA) have zoonotic importance, especially for farm workers and were also isolated from bovine mastitis in Italy and other countries[1,2]. The aim of our study was to evaluate the prevalence of S. aureus and MRSA in dairy herds and to identify the main circulating genotypes.

We examined 509 samples of bulk tank milk from eight provinces of Lombardy region. Samples were plated directly on Blood agar and the S. aureus count was determined on Baird Parker Agar + RPF. For each sample, at least 5 colonies referable to S. aureus were tested for susceptibility to oxacillin by disk diffusion test. The same milk samples were examined by subsequent enrichment in MH broth + 7.5% NaCl and TSB + 5mg/l of oxacillin and plating on Brilliance MRSA agar (Oxoid). The presence of the mecA gene was confirmed by PCR [3]. Genotyping was performed by RS-PCR (16S-23S intergenic spacer PCR) [4] and Multilocus Sequence Typing (MLST) [5].

A total of 211 out of 509 samples were positive for S. aureus (41.4%), with a prevalence in the different provinces from 9.1% to 56.8%. The counts of S. aureus showed values ranging between 10 and > 30000 cfu/ml with a median value of 100 cfu/ml. MRSA were isolated from 26 samples (5.1%). Five of these were demonstrated by both direct plating and plating after enrichment, 10 and 11 by enrichment or direct plating only, respectively. The RS-PCR identified several different profiles, including the so-called genotype B (GTB) [4] that was detected in 45 samples (21.3%). The characterization by MLST of 18 of the 26 samples positive for MRSA identified ten ST398, four ST1, three ST97 and one ST5.

The high prevalence of positive bulk milk samples confirms the importance of the S. aureus infection in Lombardy dairy farms. The analysis of circulating genotypes showed considerable variability, but it should be noted that a fairly significant rate is represented by GTB genotype which is associated with high infectivity and pathogenicity. MRSA were demonstrated in more than 10% of S. aureus positive samples, confirming the data obtained previously in the province of Lodi (personal data). The genotyping of MRSA strains showed a prevalence of typical LA-MRSA (ST398 and ST97) and ST1, the latter associated with community-acquired human infection. In the case of MRSA the application of more stringent control plans to reduce the risk of transmission to humans is recommended.

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OCCURENCE OF ORGANOCHLORINE PESTICIDES IN BOVINE FOOD CHAIN

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Nowadays, more than 800 pesticides can be found in the environment, due to a current or past use. In the present work, a multiresidues method for the simultaneous determination of 20 organochlorine pesticides (OCPs) by using GC-MS/MS was validated, in order to monitor the OCPs level in cattle feed and in bovine subcutaneous adipose tissue, with the aim to asses and verify the concentration phenomena of these persistent pollutants.

10 g of subcutaneous adipose tissue, homogenized in a cooled mixer, or finely grounded feed underwent an ultrasonic agitation with a 70 mL mixture of acetone:n-hexane (5:2, v/v) for 20 min and then by using acetonitrile.
The clean up was carried out on Florisil-SPE cartridges using 13 mL of acetone-n-hexane (1:9, v/v)
A Varian GC 3800 gas chromatograph coupled to a Varian Saturn 2000 ion trap mass spectrometer was used for the analysis and detection of the OCPs.

The OCPs residues concentrate in the adipose tissue and in blood serum of animals leading to bioconcentration and biomagnifications or accumulate in the environment because of their persistence. The pesticides most detected in animal feed were p-p' DDT, heptachlor, lindane, methoxychlor and aldrin. In subcutaneous fat sample the most detected OCPs were heptachlor, hexachlorobenzene, detected in all samples, followed by p-p' DDE, p-p'DDT, methoxychlor, lindane and p-p' DDD. Aldrin was detected in both feed samples and animal fat. The presence of aldrin in meat indicates the need for concern from the public health point of view because of its much higher toxicity than other OCPs. These results are in accordance with other authors that found most HCHS and DDTs in meat samples. In general, it was observed that the p-p' isomers of DDE, DDT and DDD were detected in samples. All detected pesticides in feed samples and fat samples did not exceed the MRLs established by the European Union.

In conclusion a rapid extraction, freezing lipid filtration and GC-MS/MS measurement methods were developed and used to measure OCPs levels in cattle feed and bovine subcutaneous adipose tissue samples to assess the possible concentration phenomena of these persistent compounds. The bioaccumulation of lipophilic pesticides like OCPs in subcutaneous adipose bovine tissue was confirmed and the feed represents a possible source of animal exposure to OCPs. The determination of pesticides residues in feed and food is therefore necessary to ensure that human exposure to these contaminants, specifically with the diet, does not exceed tolerable intakes.

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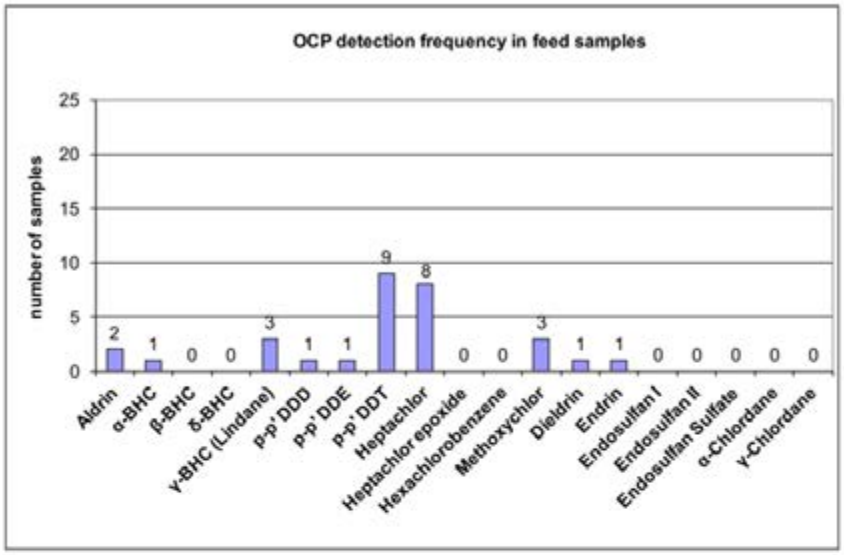
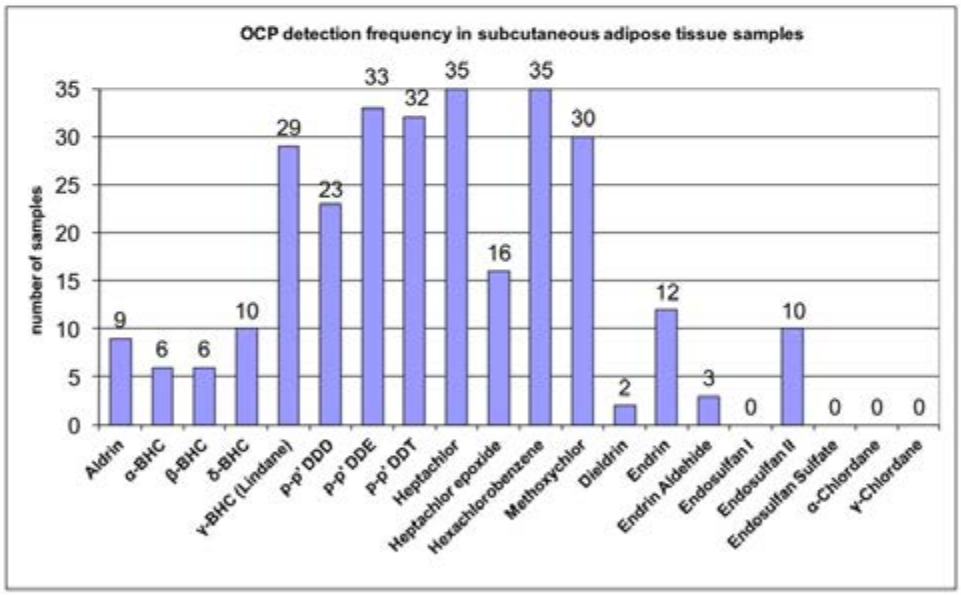
OCCURENCE OF ORGANOCHLORINE PESTICIDES IN BOVINE FOOD CHAIN



Key results about OCPs content and frequency of detection in subcutaneous tissue samples:



FAMILIAR OUTBREAK OF MONOPHASIC SALMONELLA TYPHIMURIUM TRACED BACK TO SALAMI, ITALY, JANUARY 2013

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In January 2013 a 70 years old woman died in hospital due to septicemia by group B Salmonella infection. We describe the Veterinary and Medical Public Health management of this case.

Some foods were present in the house of the victim that could be contaminated by Salmonella (1), such as homemade fresh pasta containing fresh eggs, eggs and a portion of pork salami. These left over foods were submitted to IZSLER to test for Salmonella presence and count by MPN technique. The other members of the dead woman's family were subjected to fecal examination for Salmonella.

Laboratory examination confirmed the presence of group B Salmonella only in salami. Serotyping of the isolates from the victim and salami indicated that the same serotype 1,4,12:i- was involved (monophasic Salmonella Typhimurium). The two isolates were indistinguishable by pulsed field gel electrophoresis (pulse-type STYMXB_PR.0277). Salmonella was quantified in salami at 70 MPN/g.
The fecal examinations of three family members among seven were positive for monophasic Salmonella, pulse-type STYMXB_PR.0277; only one of them had mild diarrheal symptoms. Four additional salami were present in the houses of the involved family and the same Salmonella was isolated from all of them.
The producer of the pork salami was identified by the Veterinary Health System and NAS, in an amateur producer that made those salami from a single pig slaughtered in November, 27th 2012, in an EU authorized abattoir. The salami were bought by the family around Christmas 2012.
Official control of salami that were still present in producer's warehouse confirmed the presence of the incriminated Salmonella in 4 out of 5 samples.

Considering the concentrations of Salmonella detected at different times, the traditional salami was characterized by a D reduction of Salmonella of about 28 days. That value is commonly observed in homemade salami that are added neither lactic bacteria as starter nor nitrite salts at the beginning of the production process. Given the observed D, the initial contamination of the pork meat could be estimated in about 2 Logs/g, compatible with the outcome of surveys conducted in Italian slaughterhouses (2), confirming that the risk of salmonellosis linked to the consumption of homemade pork salami have to be considered not negligible.
Moreover, this case highlights the importance of the "One health" concept and the usefulness of integrated human and animal surveillance systems in tracing food-borne disease outbreaks.

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EFFECT OF A FORMULATION OF SELECTED DAIRY STARTER CULTURES AND PROBIOTICS ON MICROBIOLOGICAL, CHEMICAL AND SENSORY CHARACTERISTICS OF SWINE NITRITE-FREE DRY-CURED SAUSAGES

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The aim of this study was the evaluation of selected lactic acid bacteria (LAB) starter culture of dairy origin in association with a commercial probiotic in the production of nitrite-free low-acid fermented pork sausage produced in a small-scale plant in Umbria (Italy), and their effect on microbiological, physico-chemical and sensorial properties of the products.

Ten salami productions in five different days were carried out in this study. Each day two different production technologies were used: without the addition of starter cultures (control) and with the addition of starter cultures (starter). Bacteriological analysis included total aerobic mesophilic microbiota, Lactococcus spp., Lactobacillus spp., enterococci, Micrococcaceae, Enterobacteriaceae, coliform organisms, Pseudomonas spp., S. aureus, sulphite reducing Clostridium spp., Clostridium botulinum, Salmonella spp., Listeria spp, non-sorbitol fermenting E. coli. With the same sampling scheme used for microbiological analysis salami were used for chemical analysis. A sensory evaluation was performed on at the end of each ripening process on control sausages and on sausages made with the addition of starter cultures.

Addition of this starter culture/probiotic formulation improved the reduction of pathogens, compared to control salami manufactured without the addition of starter cultures, yet maintaining pH and sensory characteristics well within the limit for the so-called low-acid or non-acid salami. The use of this formulation in this experiment limited the growth presumed S. aureus, enterobacteriaceae and coliform organisms and reduced the rate of isolation of S. aureus, Salmonella spp. and Listeria spp. Sulphite-reducing clostridia, Cl. botulinum and non-sorbitol fermenting E. coli were never detected in both groups

The application of the formulation of starter and probiotics strains described in this study may provide an additional tool for preventing the growth and survival of potentially pathogenic bacteria, and contribute to sensory qualities of low acid, nitrite and nitrate free, fermented sausages made with pork meat. The present study demonstrates that strains of lactococci and lactobacilli of dairy origin, along with a probiotic, is able to achieve an enhanced inhibition of pathogens and an improvement of sensory properties, and at the same time maintain the final pH within the range for non-acid/low-acid fermented sausages.

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RELATEDNESS OF STREPTOCOCCUS UBERIS MASTITIS AND ENVIRONMENTAL ISOLATES BY PULSE FIELD GEL ELECTROPHORESIS

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In this study our aim was improve our understanding of S. uberis mastitis by comparison of strains (as determined from PFGE) isolated from milk with those isolated from the environment of dairy cows where they might be exposed to this mastitis agent. Comparison of strain will allow assessment if mastitis strains are random and shared with the environment or if they are shared strains between cows and if they can be found in the environment and/or milking area.

Dairies were selected that had an ES rate of at least 10% for all culture positive samples. All ES isolates were from routine submissions of bovine milk from dairies in the central California area to the Milk Quality Laboratory, Veterinary Medicine Teaching and Research Center, Tulare, California. PFGE was performed according to standard methods. Restriction Endonuclease digestion and Pulse Field Electrophoresis: DNA in agarose gel plugs was subjected to restriction endonuclease digestion with Sma 1. Digested DNA was run on 1% agarose gels using a Chef DR II contour-clamped homogenous electric field device (Bio Rad Labs) with pulse field times of 1-20s for 20 hours at 180V for S. uberis.

Analysis of ES mastitis isolates for S. uberis by API 30 Strep to determine species indicated that dairies 1, 2, and 4 had S. uberis at 38% to 41% of all ES mastitis cases. Dairies 3 and 5 had 66% and 62% of their ES mastitis as S. uberis. PFGE analysis of mastitis isolates of mastitis cases indicated that dairies 1, 2, and 3 had multiple random strains. On dairy 4, 27 mastitis isolates were typed and 19 shared the same pulse type. The remaining 6 mastitis isolates represented 6 unique PFGE profiles. Dairy 5 had 20 mastitis isolates typed and 14 shared the same PFGE profile.

Results of this study indicate that the environmental streptococci are a heterogeneous group but when a dominant strain exists for the S. uberis on enrolled dairies, analysis of environment sources and milking equipment will help determine if shared strains are being derived from cow to cow transmission or if a more pathogenic strains of ES are residing in the environment on individual enrolled dairies.

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Pryor SM, Cursons RT, Williamson JH, Lacy-Hulbert SJ. Experimentally induced intramammary infection with multiple strains of Streptococcus uberis. J Dairy Sci. 2009 Nov;92(11):5467-75.

RISK ASSESSMENT OF PUBLIC HEALTH HAZARDS COVERED BY VISUAL INSPECTION OF SWINE MEAT: DEVELOPMENT OF A VISUAL-ONLY MEAT INSPECTION SYSTEM FOR HEAVY PIGS TOGETHER WITH A TOOL FOR THE EVALUATION OF ITS PERFORMANCES

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Back in 2011 EFSA stated that the traditional inspection system in swine is not targeted to the main hazards deriving from meat consumption. This is due to the fact that the hazards are no longer related to pathogens giving specific lesions or related to chemicals, therefore not detectable by classical meat inspection. Moreover the speed of the slaughtering lines is not easily compatible with the prescriptions of EU Regulation 854/2004. In this contest the Italian Ministry of Health, on behalf of the National Committee for Food Safety financed a project to study new inspection systems. This group developed a more risk based inspection system in heavy pigs together with a tool to assess its performances.

At first a new Food Chain Information (FCI) module was developed. The module contains information regarding the health status of flocks and also information related to the main hazards addressed by EFSA opinion. A protocol for visual-only post mortem inspection was produced together with a 24 classes scheme used to record anatomo-pathological lesions. A list of guidelines needed to get an univocal interpretation and classification of lesions was developed. A light tablet resistant to slaughter environment was commissioned to an electro medical company in order to record lesions on the slaughtering line. Considering an expected prevalence of 0.1% and a population of 8.7 million heavy pigs slaughtered in the north of Italy, in order to have a statistically significant sample 200.000 carcasses was considered an appropriate sample. In addition 40.000 carcasses were inspected to compare traditional and visual inspection. A number of 5 veterinaries were chosen and trained to follow the guidelines for the recording of lesions. Their performances were tested along the project.

The FCI module contains the health status regarding the following: swine vesicular disease, swine fever virus, tuberculosis, brucellosis, trichinella, erysipelas, Aujeszky, PCV2, PRRS. It also contains the record of respiratory, reproductive and enteric symptoms of the animals. It is asked if the farm in the last 90 days tested positive for the following pathogens: E. coli, Leptospira interrogans, Lawsonia intracellularis, Salmonella spp., Influenzavirus A, Mycoplasma hyopneumoniae. Finally the breeder is asked of records of lesions at slaughter in the previous slaughtering. The list of lesions developed in collaboration with the veterinary services of Emilia-Romagna and Lombardy is presented in Table 1.

The output of the project is a new visual-only meat inspection system which is more risk based and able to cope with the needs of a high intensity pig production chain such as the ones of the North of Italy. This system also contains instruments useful for the evaluation of its performances.

• EFSA (2011) EFSA Journal. 2011; 9(10), 2351 [198 pp.];
• Regulation (EC) No. 854/2004. Official Journal of the European Union, L155, 206. 29 April 2004.

APPARATUS	LESION
Respiratory	pneumonia pleuropneumonia
Digestive	hepatitis hepatosis/hepato dystrophies peritonitis/perihepatitis enteritis
Reproductive-Urinary	nephritis nephrosis cryptorchidism
Cardio Circulatory	myocarditis pericarditis
Integumentary	dermatitis erysipelas
Loco motor	arthritis muscle color alteration (PSE/DFD) oedema/emaciation
Other (carcass)	jaundice abscesses neoplasms / tumors biliary or faecal contamination trauma lymphadenopathy splenomegaly petechial haemorrhages

COMPUTERIZED JAVA-BASED TOOL FOR ON-FARM ANIMAL WELFARE AUDIT FOR SHEEP AND GOATS

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The establishment of an audit checklist adapted and developed for dairy goats can be used to monitor welfare. In similar studies the relationship between the cows' lameness and the milk production is highlighted: cows with a higher incidence of locomotion problems had lower milk production.

A checklist for the audit was developed to measure the following parameters: body condition score, cleanliness of water, cleanliness of the udder, space allowance, locomotion scores, hoof care, integument alterations, coughing animals, nasal discharge, diarrhea, disbudding/dehorning, agonistic behavior, avoidance distance, exploratory behavior. A Java software has been developed to calculate farm score for animal welfare based on the measurements of the checklist. The total score is the result of the calculation of the weighted average of the four main categories: Good Feeding, Good Housing, Good Health and Appropriate behavior.

Data for a single farm are provided as an example.
Total score for GOOD FEEDING main category: 73.33
Total score for GOOD HOUSING main category 93.39 points
Total score for GOOD HEALTH main category: 72.57 points
Total score for APPROPRIATE BEHAVIOR main category 57.29 points.
Total Farm Score: 73.65 points

The check list was easy to use and could be exploited by farm owner or manager to monitor his farming as practices an internal audit. It often happens that the condition in a farm deteriorate without noticing: an audit check list could be periodically used to see if welfare and management are under control.
There is increasing international interest, proven also by the intervention of OIE and by the he Commission of the European Communities which focused part of its programme on animal welfare legislation, relations with third countries and the implication for the EU. An audit about livestock welfare could help to maintain the required standards of welfare.

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2: de Vries M, Bokkers EA, Dijkstra T, van Schaik G, de Boer IJ. Invited review: associations between variables of routine herd data and dairy cattle welfare indicators. J Dairy Sci. 2011 Jul;94(7):3213-28. doi: 10.3168/jds.2011-4169. Review. PubMed PMID: 21700006.

3: Rushen J, Butterworth A, Swanson JC. Animal behavior and well-being symposium: Farm animal welfare assurance: science and application. J Anim Sci. 2011 Apr;89(4):1219-28. doi: 10.2527/jas.2010-3589. Epub 2011 Jan 7. PubMed PMID: 21216980.

ANTIMICROBIAL RESISTANCE OF STHAPYLOCOCCUS AUREUS ISOLATED FROM FOOD HANDLERS

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Staphylococcus aureus (S. a.) is one of the leading causes of all food-borne illnesses worldwide. The aim of this study is to assess the prevalence of S. a. strains in food handlers and their profile of antibiotic resistance.

N. 221 samples were collected. Isolation of S. a. was carried out according to UNI EN ISO 6888-2. Confirmation was then conducted using a multiplex PCR for the detection of the 16S rRNA and the nuc genes. Each strain was tested against 16 antimicrobial agents using Vitek system (GPS 538 Card). In addition the strains were subjected to a PCR for the detection of mecA gene.

S. a. was present in 29 samples (13.1%). Of them, 65.5% (19/29) was sensitive to all the tested antibiotics; 10.3% (3/29) had an intermediate susceptibility to ciprofloxacin, norfloxacin and fosfomycin; 24.1% (7/29) was resistant. In particular, among the resistance strains, 57.1% (4/7) demonstrated multi-resistance to three or more antibiotics. The most frequent antibiotic resistance observed was for ampicillin/sulbactam, cephalothin and oxacillin. All strains tested showed susceptibility to Vancomycin, Gentamicin, Fusidic Acid and Nitrofurantoin.A total of 4 isolates (13.8%) were MRSA. Interestingly, n. 2 strains (6.9%) harboured the mecA gene. Among them, one showed multi-resistance to 5 antibiotics (ampicillin/sulbactam, cephalothin, oxacillin, clindamycin and tetracycline) and one showed an intermediate susceptibility to fosfomycin.

There is clear evidence that the intensive use and misuse of antimicrobial drugs leads to a selection of resistant microorganism. The percentage of resistance to ampicillin, cephalothin and oxacillin reflects the widespread use of these antibiotics in human and veterinary therapy. Different rates of MRSA have been reported in other studies. Paludi et al. found that the occurance of MRSA was 1.8% while Loeto et al. reported a rate of 22.4%. Among the MRSA isolated, 2 strains were mecA positive and one of these was phenotipycally susceptible to oxacillin while 2 strain were mecA negative but phenotipycally resistant. Although the presence of mecA gene is an important mechanism of methicillin resistance, other factor alone or in association can be involved. The presence of MRSA strains emphasized the role of humans as an important reservoir of MRSA and highlighted the need for a better sanitary education of food handlers focusing on their potential role as spreaders of foodborne pathogens.

Loeto D., Matsheka M.I., Gashe B.A. (2007). Enterotoxigenic and antibiotic resi stance determination of S. aureus strains isolated from food handlers in Gaborone, Botswana..J Food Prot. 70(12):2764-8. Paludi D., Vergara A., Festino A.R., Di Ciccio P., Costanzo C., Conter M., Zanardi E., Ghidini S., Ianieri A. (2011). Antimicrobial resistance pattern of methicillin-resistant S. aureus in the food industry. J. of Biol. Reg. & Omeost. Agents.V. 25, n.4, 671-677.

SPREAD OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN TWO FARMS WITH DAIRY COWS AND PIGS AT CLOSE CONTACT

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The aim was to identify through an epidemiological molecular survey the likely source of mammary infection due to MRSA in two farms linked to pig farming. Isolates from milk, environment, animal and human nasal cavities were typed in order to trace a pathaway of contamination or infection in each farming system.

From February to June 2010 all Staphylococcus aureus isolates from cow mastitis were screened for resistance to oxacillin and in case confirmed MRSA by PCR. In the MRSA positive farms a survey was conducted collecting MRSA from nasal cavity of humans and animals within and around the farms and in environmental dust. All isolates were typed by MLST and spa-typing.

A total of 183 Staph. aureus were screened in 27 dairy farms. Both farm A and B which resulted positive were strictly related to a pig holding. Farm A was under the same property of a farrow-to-finish pig holding. Farm B was near a fattening pig holding. No MRSA negative dairy farm had relation with pig farming. Typing of 23 isolates from milk, 9 from human nasal cavity, 8 from pig nasal cavities and 15 from environment was performed. ST398 was the most common type among milk isolates (13/23), other types detected were ST97 t4795 (8/23) and ST97 t1730 (2/23). Among isolates from dust ST398 t899, ST1 t127, ST97 t4795 and t1730 were found. In particular, ST97 t1730 was detected in dust from milking parlor in farm B. All the types were detected in nasal cavities of pigs, but ST1 t127. Nasal swabs from milkers/owners in farm A yielded ST398 t899 and ST974795, whereas milkers in farm B were negative at time of sampling.

A heterogeneity of ST and spa types was demonstrated confirming the findings of Battisti et al. and of EFSA report (2010) in Italian pig population. Types were found in different specimens, included human nasal cavities denoting a wide and complex MRSA circulation, likely from pigs that are known reservoir (Broens et al., 2010) to cows, in which MRSA can cause mastitis (Spohr et al., 2010). Humans can be transiently infected and represent a vector, especially during milking. Moreover, milking parlor dust can be a source of MRSA. The results advice to implement strict hygienic procedure to prevent contamination and infection in dairy herds at risk due to proximity to pig holdings.

Battisti, A. et al., 2010. Heterogeneity among methicillin-resistant Staphylococcus aureus from Italian pig finishing holdings Vet. Microbiol. 142:361–366
Broens, E.M. et al., 2010. MRSA CC38 in the pig production chain. Preventive Veterinary Medicine 98 (2011) 182–189
EFSA, 2010. Scientific report of EFSA. Analysis of the baseline survey on the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in holdings with breeding pigs, in the EU, 2008. Part B: factor associated with MRSA contamination of holdings. EFSA Journal 8(6):1597
Spohr, M. et al., 2010. Methicillin-Resistant Staphylococcus aureus (MRSA) in three dairy herds in Southwest Germany. Zoonoses and Public Health

QUALITY INDEX METHOD (QIM): DEVELOPMENT OF A SENSORIAL SCHEME FOR MANTIS SHRIMP (SQUILLA MANTIS)

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Mantis shrimps (M.S.) are one of the species most valued and marketed in the Adriatic regions. Given the shortcomings of current legislation, which are not reported methods or parameters for the evaluation of the freshness, is strongly felt the need to perfect ways to assess the organoleptic characteristics without the aid of analysis laborious in terms of time, cost and labor.

Five batches of M.S. were purchased directly from a local fisherman at the port of Giulianova (Teramo). At the laboratory M.S. were kept in boxes with perforated bottoms to allow drainage of melted water, at refrigeration temperatures (2±2 °C). Ice was added to the boxes every day. A total of 250 whole raw M.S. with an average weight and length of 50 g and 18 cm were used to design the QIM table. Three assessors described day-to-day changes that occurred during storage in 5 independent degradation experiments. Sensorial analysis were performed in triplicate every day until time of sensory rejection. Data obtained were submitted to time-dependent linear regression analysis. The equation which was best fit and the correlation coefficient (R2) between the Quality Index (QI) and the storage time in ice were also calculated. Results obtained from QIM were then submitted to partial least-squares regression to estimate the uncertainty (standard error of estimate) of the prediction of the QI.

The QIM scheme presented proved to give a good description of the changes in whole raw M.S. during ice storage. The final QIM scheme developed for the M.S. included 8 parameters with a total of 14 demerit points (table 1). Average QI showed an increasing linear trend and were highly correlated (R2=0.954) with time of storage. PLS model applied to data indicated that the regression model proposed had a standard error of estimate of approximately 1 day. Most important attribute, as reported by Bremner (1998), was appearance (R2=0.96). The other parameters showed excellent correlation indices with the exception of head and uropods who showed values of 0.89 and 0.80, respectively. Rejection of mantis shrimp is considered to occur at day 7.

The QIM scheme as presented in this article is the first of a series of steps. As mentioned by Larsen (1997), QIM is a method that implies the transformation of scientific knowledge of the products in a consumer friendly solution that can be used by the fish retailer and the consumer in common, which is both rare and desirable.

Bremner A. (1998). If freshness is lost, where does it go? In Methods to determine the freshness of fish in research and industry. Int. Inst. Of Refrigeration, p. 36-51; Larsen, E. (1997). Transformation of scientific knowledge about quality for practical use in the Danish fish sector. In Methods to determine the freshness of fish in research and industry. Int. Inst. Of Refrigeration, p. 319-324.

Parameter	Description	Demerit point
Appearance	Shiny, with no black spots	0
	Less shiny to matt	1
	Matt, black colour between the segments of the carapace and black colour	2
Head	Adherent, no black spot	0
	Less adherent, light gray color	1
	Loosened, black color	2
Eyes	Shiny, bright green, smooth	0
	Slightly matt, green/gray, smooth	1
	Matt, yellow/brown, rougher	2
Odor	Seaweed, no off-odor	0
	Neutral, species specific, slightly off odors	1
	Stale, off-odor	2
Tail	Shiny, noticeable purplish edges, no black spots	0
	Less shiny, pale purplish edges, no black spots	1
	Matt, pale purplish edges or absent, noticeable black spots	2
Uropods	Shiny, characteristic color	0
	Dull, bleached	1
	Dull, bleached, noticeable black spots	2
Pleiopods	straw yellow, transparent	0
	straw-yellow or white/gray, translucent	1
	white/gray, noticeable black spots	2
Quality Index		0-14

SPECIES IDENTIFICATION IN PETFOODS BY USING BLAST ANALYSIS OF A FRAGMENT OF THE MITOCHONDRIAL 16S RIBOSOMAL RNA GENE (16SRRNA).

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The aim of this work was to verify the label information of pet food for cats, reporting the commercial denomination of Bianchetto among the ingredients, by sequencing and BLAST analysis of a short fragment of the mitochondrial 16SrRNA gene

Fifteen samples of petfood for cats were collected from the retail market. Three fish per samples were analyzed. After DNA extraction, performed according to Armani et al. (2011) (1), the DNA degradation pattern was assessed by electrophoretic analysis. On the basis of the 108 sequences available in GenBank belonging to the order Clupeiformes and Osmeriformes, four different primers were designed and used in combination with those reported in Armani et al. (2012) (2), for the amplification of short fragments of the mitochondrial 16SrRNA gene with a length ranging from 77 to 246 pb. Such fragments were preventively tested by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome) to assess their discriminatory power at inter and intra specific level. After PCR amplification, the samples associated to the expected amplicon were sequenced and the 45 sequences obtained were analyzed using the program Clustal W in Bioedit version 7.0.9.0 (3) and identified by BLAST analysis.

The BLAST analysis returned an identity values of 100% with different species of the genus Encrasicholina. In particular, the most part of the samples were identified at the species level as E. heteroloba, and E. punctifer.

The obtained results confirms that the molecular marker selected in this study can be used for the identification of species belonging to the Clupeiformes order, allowing a discrimination even among close species. All the 15 market pet food samples were mislabeled. In fact, while the species identification performed by molecular analysis clearly showed the presence of the juvenile form of Encrasicholina sp, also known as tropical anchovies, all the labels reported the commercial denomination of Bianchetto, which, in Italy, is allowed only for the juvenile form of Sardina pilchardus (4).

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INTESTINAL AND PULMONARY PARASITES IN STRAY DOGS AND CATS IN THE CITY OF NAPLES

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The present study reports the results of a survey on the presence and distribution of intestinal and pulmonary parasites in stray dogs and cats in the city of Naples.

A total of 2828 faeces from stray dogs and 1176 faeces from stray cats were collected at the Regional Center for Urban Veterinary Hygiene from 2009 to 2013, located in Naples (southern Italy) and analyzed to evaluate the presence of intestinal and pulmonary parasites. On each faecal sample, copromicroscopic analyses were performed using the FLOTAC dual technique (Cringoli et al., 2010) having an analytic sensitivity of 2 eggs/larvae/oocysts/cysts per gram of faeces (EPG/LPG/OPG/CPG). A sodium chloride-based flotation solution (FS2, specific gravity (s.g.)= 1.20) and a zinc suphate-based flotation solution (FS7, s.g. = 1.45) were used.

The results of the survey are summarized in table 1 (dogs) and table 2 (cats).

The present study showed the presence of endoparasites in 59.5% of dogs with a prevalence higher in male subjects < 8 months. Regarding stray cats 69.4% of animals analyzed had at least one parasite, with higher prevalence in cats < 8 months.
The prevalence values detected in the present study were slightly higher than those previously conducted on dog faecal samples from the city of Naples (40.6%) (Rinaldi et al., 2006), from the Benevento province (55.8%) (Musella et al., 2010) and from kennel dogs distributed in the Campania region (Rinaldi et al., 2011). This is the first survey on parasites in stray cats performed in the Campania region.
In conclusion, the results of the present study showed the presence of canine and feline parasitic elements, some of which are agents of zoonosis (i.e. T. canis, Ancylostomidae, T. vulpis and Taenia spp.). It is desirable to plan such parasitological investigations also on a large scale, using advanced epidemiological and diagnostic methods in order to better target effective control strategies.

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Table 1. Prevalence (%) of parasites detected in the 2828 stray dogs analyzed in the city of Naples.

		Sex		Age	
		Female	Male	<=8months	> 8 months
Parasites	Dogs (n = 2828)	(n =1389)	(n = 1439)	(n = 672)	(n = 2156)
Endoparasites	59.5	57.7[b]	61.2[a]	65.8[a]	57.6[b]
Toxocara canis	25.8	28.6[a]	23.1[b]	44.5[a]	20.0[b]
Toxascaris leonina	1.2	1.6[a]	0.8[a]	1.0[a]	1.3[a]
Trichuris vulpis	26.3	27.5[a]	25.2[a]	9.2[b]	31.7[a]
Ancylostomidae	16.0	18.4[a]	13.4[b]	11.7[a]	1.7[b]
Strongyloides stercolaris	0.1	0.1[a]	0[a]	0[a]	0.1[a]
Angiostrongylus vasorum	0.5	0.7[a]	0.4[a]	0.3[a]	0.6[a]
Crenosoma vulpis	0.1	0.1[a]	0.1[a]	0.3[a]	0.1[a]
Oslerus osleri	0.6	0.7[a]	0.5[a]	0.3[a]	0.7[a]
Dipylidium caninum	4.9	5.3[a]	4.5[a]	3.9[a]	5.2[a]
Taenia spp.	0.1	0.1[a]	0[a]	0[a]	0.1[a]
Isospora canis	11.2	11.7[a]	10.8[a]	17.1[a]	9.4[b]
Isospora ohiensis	1.6	1.7[a]	1.6[a]	1.8[a]	1.6[a]

Significant differences for different letters (P<0.05).

Table 2. Prevalence (%) of parasites detected in 1176 stray cats analyzed in tha city of Naples.

		Sex		Age	
		Female	Male	<=8months	> 8 months
Parasites	Cats (n = 1176)	(n =595)	(n = 581)	(n = 568)	(n = 608)
Endoparasites	69.4	69.4[a]	69.4[a]	82.2[a]	57.4[b]
Toxocara canis	47.6	46.2[a]	49.1[a]	62.1[a]	34.0[b]
Toxascaris leonina	0.1	0[a]	0.2[a]	0.2[a]	0[a]
Ancylostoma tubaeforme	2.1	2.4[a]	1.9[a]	0.5[a]	3.6[b]
Dipylidium caninum	1.4	1.5[a]	1.4[a]	1.2[a]	1.6[a]
Aelurostrongylus abstrusus	15.6	15.8[a]	15.3[a]	13.2[a]	17.8[b]
Isospora felis	34.4	36.1[a]	32.7[a]	48.1[a]	21.7[a]
Isospora rivolta	3.9	2.7[b]	5.2[a]	5.5[a]	2.5[b]

Significant differences for different letters (P<0.05).

CHARACTERIZATION AND MODELING OF THE GASTROINTESTINAL PARASITIC FAUNA IN ALPINE MARMOTS MARMOTA MARMOTA

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The Alpine marmot Marmota marmota is considered an "extreme" animal, due to its capacity of surviving in severe mountain habitats. The center-point of its life cycle is hibernation, whose success depends on the amount of energy stored during the previous summer. Intestinal parasites, when present in high numbers, can compromise the accumulation of reserve-fats and hibernation itself (Callait and Gauthier, 2000). The goal of this project was to characterize the parasitic community of alpine marmots, its abundance and to model it from an environmental point of view.

We collected and analyzed for an entire season of activity (from April to September 2012) fecal samples of marmots (one sampling every two weeks) living within the entire altitudinal range of the species in the Gran Paradiso National Park (Aosta Valley, Italy). Fecal samples (n=605) were analyzed by qualitative and quantitative (McMaster) coprological techniques. Inference on distribution of parasites was done using an Occupancy model (Fiske and Chandler, 2011) which assumes that the probability of detecting a species is directly linked with its abundance. Samples were classified according to altitudinal range (n=6 areas of ~ 200 m of eight difference, from 1700 to 2800 m.a.s.l.), yearly mean solar radiation (kW/h) on the sampling point, and by date.

We identified a cestode (Ctenotaenia marmotae) and a nematode (Ascaris laevis), together with three species of coccidia of the genus Eimeria. C. marmotae detection probability was inversely correlated to altitude ($p<0.001$) and solar radiation ($p<0.05$), while its abundance progressively increased in time ($p<0.001$). The resulting model expained 85% of the total variance of data. A. laevis detection probability was affected by time of sampling ($p<0.001$) and altitude ($p<0.001$). The model with these two covariates explained 91% of the total variance of the dataset.

Our data suggest that environmental factors deeply influence parasite abundance in marmots. Solar radiation influences C. marmotae detection because it affects its intermediate host (mite of Oribatidae family) while altitude influences both C. marmotae and A. laevis. We can speculate that marmots that "choose" to live in less favorable habitats (i.e. higher altitudes) can gain the same amount of fat-reserves and successfully overwinter, like marmots living in more favorable conditions (i.e. lower altitudes) because they are infested with a lower number of parasites.

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TOXOPLASMA GONDII IN PIEDMONT: EPIDEMIOLOGY IN WILDLIFE AND LIVESTOCK.

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Toxoplasma gondii is an apicomplexan parasite able to infect almost all warm blooded animals, that has the domestic cat as main definitive host. Worldwide, 6 billion people are infected with T. gondii (Furtado et al., 2011). In Piedmont (Northwestern Italy), 0.1% of women seroconvert during pregnancy each year (Pezzoli et al., 2009). The goal of our research is to evaluate prevalence of infection with T. gondii in domestic and wild animals from Piedmont.

We sampled skeletal muscle (SM) and encephalon (CNS) from 355 wild animals (n= 121 roe deer Capreolus capreolus, n= 105 wild boar Sus scrofa, n= 94 red fox Vulpes vulpes, n=22 chamois Rupicapra rupicapra, n= 13 red deer Cervus elaphus), from 89 Piemontese cattle and from 90 pigs born and raised in Piedmont. From cattle and pigs we also collected blood that was tested by ELISA. All tissue samples were tested for T. gondii by PCR (Homan et al., 2000).

We found T. gondii with an overall prevalence of 12.17% (IC 95% 0.1-0.15). For wildlife (P=10.99% IC 95% 0.08-0.15), we report a higher rate of infection in carnivores and omnivores (P=20.0%, IC 95% 0.13-0.29 in fox; P=16.0%, IC 95% 0.1-0.24 in wild boar) compared to ruminants (P= 2.5%, IC 95% 0.009-0.074 in roe deer; P= 0.0% in red deer - IC 95% 0.00- 0.23 and chamois - IC 95% 0.00-0.15). In cattle prevalence was 14.61% (IC 95% 0.09-0.23) and 14.44% (IC 95% 0.09-0.23) in swine. ELISA compared to PCR has a sensibility of 50% and specificity of 96% in cattle, while in pigs of 86% and 95% respectively.

T. gondii is present in Piedmont with similar prevalences in wildlife and livestock. Among wildlife, carnivores and omnivores are significantly more exposed to T. gondii infection than herbivores, suggesting the importance of tissue cysts in spreading the disease (Smith and Frenkel, 1995). The prevalence reported in livestock, can be explained by the possible presence of cats on farms and by contamination of water sources with oocysts. Considered the low sensibility of serology on tested animals, our data suggest that to meet the growing need of having T.gondii-free meat, PCR could be an efficient diagnostic method.

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HEPATOZOON CANIS, SPIROCERCA LUPI AND PHYSALOPTERA SIBIRICA IN RED FOX VULPES VULPES FROM NORTHWESTERN ITALY

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In order to fill the lack of information on the epidemiology of Hepatozoon canis, Spirocerca lupi, and Physaloptera sibirica in Northern Italy, we investigated the presence of these parasites in wild red foxes Vulpes vulpes from Piedmont Region. In the region, as elsewhere, the population of wild foxes is constantly growing, moving closer to urban settlements. This expansion makes contacts with domestic animals and humans more frequent and wildlife becomes valuable sentinel host to monitor the distribution of diseases.

We analyzed foxes found dead or culled within the official regional culling programs coming from the provinces of Torino, Biella, Cuneo, Novara and Alessandria (Piedmont region). Each fox was necropsied following standard procedures. Age was determined on the basis of dental eruption/consumption, and for each fox date and place of culling was recorded. The stomach content, washed and filtered was inspected for the presence of P. sibirica and S. lupi. The presence of H. canis DNA was assessed on spleen samples, using a specific PCR targeting a 670bp fragment of the 18S SSU rRNA gene (Baneth et al., 2000).

We analyzed 910 foxes from 2010 to 2013. P. sibirica was found in the stomach of 5 foxes (p= 0.55%; IC95% 0.0023-0.0128), while S. lupi was found in 5 different individuals (p= 0.55%; IC95% 0.0023-0.0128). H. canis DNA was detected in the spleen of 10 foxes out of the 324 analyzed (p= 3.09% IC95% 0.0168-0.0559).

This is to our knowledge, the first report of H. canis and S. lupi from Northwestern Italy. P. sibirica was reported in foxes and badgers for Piedmont with slightly higher prevalence in 2009 by Ferroglio and colleagues, as well higher prevalences were previously reported for S. lupi in Sicily (p=9.16%) (Ferrantelli et al., 2010) and for H. canis (p= 13.4%) (Gabrielli et al., 2010).

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INTESTINAL PARASITES IN KENNEL DOGS IN NORTH-EASTERN ITALY

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The aim of this survey was to consider the parasitological conditions of kennel dogs in North-eastern Italy, evaluating presence and prevalence of intestinal parasites, including Giardia spp. and Cryptosporidium spp., and correlating them with potential risk factors.

From November 2008 to June 2012, 318 stool samples were collected in 8 different kennels (7 in Veneto and 1 in Friuli Venezia Giulia regions). Individual data were collected about breed (n=265), sex (n=213), age (n=189) and anthelmintic treatment within 2 months before sampling (n=162). Firstly, all the faecal samples were checked for parasites with a standard copromicroscopic technique and then 296 out of them were subjected to PCR to detect Giardia spp., and (still in progress) Cryptosporidium spp. Molecular analysis for Giardia included a nested-PCR targeting the 16S gene (Read et al., 2002). Amplicons were sequenced and compared with sequences available in GenBankTM. Differences in parasite prevalence in relation to dogs' individual data were evaluated using Pearson's Chi-squared Test (significance level $p<0.05$). Parasites with prevalence values below 6% were not considered.

A total of 167 (52.5%) out of 318 faecal samples, tested with the copromicroscopic technique, were positive. The most prevalent helminths were T. vulpis (29.2%), T. canis (9.7%), and A. caninum (8.2%); among protozoa, Giardia reached the highest prevalence value 15.1% (Table), that increased to 39.5% (117/296) using PCR. Sequencing is still ongoing (at present carried out on 60/81 PCR positive samples). G. duodenalis assemblage C was found in 41 samples, assemblage D in 18 and B1 only in one. The provenance kennel was influencing the prevalence of nearly all considered parasites (Table), while breed, sex and age were not. The prevalence of T. vulpis and A. caninum were significantly lower ($p<0.01$) in treated animals (11.7% vs. 57.8% and 3.3% vs. 20.6%, respectively).

This survey confirms that dogs, kept in a limited area, have high probabilities to have helminthic and/or protozoan infections and significant variations in parasite prevalence occur in relation to the management of each kennel and anthelmintic treatments. Trichuris and Giardia are confirmed as the most prevalent intestinal parasites in kennel dogs, in agreement with previous studies (Capelli et al, 2006). However, molecular tools are preferably required to detect Giardia parasite. The results confirm dog as a possible host for Giardia assemblage B, cluster B1, already isolated in human beings (Lalle et al, 2005); the zoonotic role of this parasites is not confirmed and further studies are needed.

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Parasite	Veneto							FVG*	Total (n=318) Pos (%)	χ^2	P value
	Bassano (n=26) Pos (%)	Vicenza (n=47) Pos (%)...	San Donà (n=52) Pos (%)	Treviso (n=17) Pos (%)	Piazzola sul Brenta (n=38) Pos (%)	Rovigo (n=26) Pos (%)	Verona (n=24) Pos (%)	Udine (n=88) Pos (%)			
Trichuris vulpis	7 (26.9)	18 (38.3)	37 (71.2)	1 (5.9)	10 (26.3)	4 (15.4)	2 (8.3)	14 (15.9)	93 (29.2)	65,757	p<0.001
Toxocara canis	4 (15.4)	13 (27.7)	0	0	0	2 (7.7)	0	12 (13.6)	31(9.7)	33,863	p<0.001
Ancylostoma caninum	0	3 (6.4)	20 (38.5)	1 (5.9)	1 (2.6)	0	0	1 (1.1)	26 (8.2)	77,981	p<0.001
Eucoleus aerophilus	0	0	0	0	0	0	0	7 (7.9%)	7 (2.2)	-	-
Dypilidium caninum	0	0	2 (3.8)	0	0	1 (3.8)	0	2 (2.3)	5 (1.6)	-	-
Giardia spp.	13 (50)	13 (27.7)	2 (3.8)	0	4 (10.5)	7 (26.9)	8 (33.3)	1 (1.1)	48 (15.1)	61,728	p<0.001
Coccidia (*Isospora* spp.)	0	0	2 (3.8)	1 (5.9)	0	2 (7.7)	4 (16.7)	9 (10.2)	18 (5.7)	-	-

Table. Results of copromicroscopic technique and prevalence (%) of parasites in 8 different kennel. *Friuli Venezia Giulia

ANTI-TOXOPLASMA GONDII ANTIBODIES IGG AND IGM IN HEAVY PIGS REARED IN NORTHERN ITALY

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The aim of this study was to estimate the T. gondii seroprevalence in heavy pigs reared in high containment level farms of Northern Italy and slaughtered at 9 months of age.

Blood samples were collected from 20 heavy pigs for each of 125 originating from 125 herds at the slaughterhouses from 2010 to 2012. T. gondii specific IgG and IgM antibodies were detected by two commercial competitive ELISA ID Screen Toxoplasmosis Indirect Multi-Species (ID VET innovative diagnostics). The ELISA results were interpreted according to the manufacturer's instruction as: negative, inconclusive (borderline), positive, and positive/acute infection. When at least one blood sample per batch resulted as "positive" or as "positive/acute" by the ELISA, the whole batch was considered as "positive".

". Anti T. gondii antibodies were detected in 111 (4,44%) out of 2500 pigs. According to the manufacturer's instruction, 30 (24%) herds resulted positive and 4 (3,2%) herds resulted "positive with acute infection". In 20 (16%) herds, positive animals ranged between 1 and 2, in 3 (2,4%) between 3 and 5, and in 3 (2,4%) >5.

The results of the present study show that the T. gondii seroprevalence in heavy pigs reared in Northern Italy was relatively high, even if the acute infection occurs only sporadically. The collection of data on feed, environmental and management conditions at the herd level will be useful to evaluate the risks of T. gondii transmission, to improve the integrated measures and reduce the seroprevalence in the heavy pig production of Northern Italy.

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SEROPREVALENCE OF EHRLICHIA CANIS AND RICKETTSIA CONORII IN DOGS IN SICILY

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Canine vector-borne diseases (CVBDs) comprise a group of infectious diseases caused by several pathogens (bacteria, protozoa, and helminths), which are transmitted by arthropod vectors. As some CVBD-causing pathogens (A. phagocytophilum, Bartonella spp., E. canis, L. infantum, Rickettsia spp.) are of major zoonotic interest, these diseases constitute an emerging worldwide public health threat for pet dogs and their owners.Canine ehrlichiosis and rickettiosis are diseases transmitted by Rhipicephalus sanguineus ticks and caused by obligatory intracellular bacteria like Ehrlichia canis and Rickettsia conorii. These pathogens are transmitted to humans principally by infected arthropods, but contamination by aerosol and blood transfusion has also been described. The aims of this study are to determine the seroprevalence of E.canis and R. conorii and to evaluate their geographical spread in Sicily.

Serum of 1751 and of 1281 dogs were analysed for E.canis and R. conorii respectivetly. Indirect Immunofluorescence Assay (IFA) was carried out on all serum samples to analyze the presence of antibodies against R. conorii and E.canis.

IFA analysis revealed that R. conorii (69,3%) is the most prevalent infective pathogen when compared to E. canis (35,3%). Palermo, Agrigento, Messina and Trapani are the cities with a greater number of serologically R. conorii positive. Most E. canis positive samples were found in Palermo, Caltanissetta and Messina.

In the Mediterranean area, R. conorii is the primary spotted fever agent while Ehrlichia spp. is considered the agent of emerging tick-borne disease in humans and animals (dogs and livestock). Tick ecology determines all the epidemiological aspects of tick-bite fevers. In conclusion, in areas of endemicity, the diagnosis of CVBD infections depends on a wide range of factors, including animal exposure to arthropod vectors and the immunopathogenesis of infection among individual animals, which can be influenced by a variety of other host-related factors.

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RISK OF TICK-BORNE ZOONOSES IN NORTHEASTERN PIEDMONT, ITALY

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Tick-Borne Zoonoses (TBZ) are widespread in many European countries and their incidence has increased over the past few years. Their geographical distribution is still expanding both toward higher altitudes and latitudes. Domestic and wild vertebrates play an important role in promoting the spread of the vector and the pathogen in the territory; the man is usually only an occasional host. TBZ risk is linked to tick abundance, and the tick exposure increases in the case of particular occupational (forestry work and farming) or leisure activities (hunting, mushroom collecting and berry picking) (Alciati et al. 2001). Since 2009 tick-bite events are increasingly reported by the local population in Verbano-Cusio-Ossola (VCO) Province, northeastern Piedmont, where 7 clinical cases of Borreliosis were officially diagnosed between 2010 and 2011. The aim of this study was to investigate tick fauna and tick infection by pathogens in this province.

Sites located at different altitudes were selected and questing ticks were monthly collected by dragging from April to September 2011-2012. Ticks from bitten patients were also investigated.

A total of 3224 ticks (2514 larvae, 693 nymphs, 17 adults) were collected and identified as Ixodes ricinus. Most of the questing ticks were found at an altitude between 1000 and 1200 m above sea level. Larvae peaked in May, while nymphs were more abundant in summer; few adult ticks were collected. Ticks collected on humans were overall 96 (4 larvae, 61 nymphs, 31 adults); 75% of these were I. ricinus, 20.9% Ixodes spp., 1% Ixodes hexagonus and 3.1% Rhipicephalus sanguineus. Preliminary tests targeting Borrelia burgdorferi s.l., Rickettsia spp., Anaplasma spp. showed infection prevalences of 10.4%, 4.3% and 1.5% respectively in questing ticks and prevalences of 4.5%, 16% and 3.4% respectively in ticks from humans. No infection by Tick Borne Encephalitis virus (TBEV) was found.
Borrelia positive samples were sequenced, and four genospecies were found: B. afzelii, B. garinii, B. valaisiana and B. lusitaniae. Finally, phylogenetic analysis based on the OspC gene showed that most of the strains from pathogenic genospecies might have the potential for human infection and for secondary invasion.

An abundant I. ricinus population was registered. This is the most common tick species in Europe and the vector of several zoonotic agents. TBZ in humans are associated with local tick abundance, density of vertebrate reservoir hosts, climate changes and ticks infection prevalence. The analysis of these factors can help in assessing risks and to guide the implementation of public health policies against TBZ (Matassa 2007).

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A PROMISING NEW ELISA DIAGNOSTIC TEST FOR CATTLE BABESIOSIS BASED ON BABESIA BIGEMINA APICAL MEMBRANE ANTIGEN-1.

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The babesiosis due to Babesia bigemina is a relevant constraint worldwide. The tick-borne pathogen infects bovine and causes a severe disease, strongly reducing meat and milk production. For vaccine and diagnostic purposes, many surface antigens of the pathogen have been analyzed, among these, the recently identified (1) Apical Membrane Antigen 1 (AMA-1). This study is focused on the B.bigemina AMA-1 and on its use for the assessment of a diagnostic test.

AMA-1 sequence was amplified from a B.bigemina Italian strain, cloned into an expression vector and the plasmid was used to transform E.coli competent cells. Protein expression was induced by IPTG 0.75 mM for 2 hours at 37°C. After polyacrylamide gel electrophoresis, recombinant protein was visualized on UV light thanks to the presence of a Lumio™ Tag codified by the vector sequence. Bacterial cells were sonicated and the recombinant protein purified by histidine-tag chromatography. After enzymatically cleavage of the tag, the protein was quantified by spectrophotometer. The protein, diluted in a carbonate buffer, was used for the adsorption to the ELISA plate well (Maxisorp®, Nunc) over night at 4°C (2).
Italian field sera positive to B.bigemina were used to evaluate the presence of anti AMA-1 antibodies. In order to verify the specificity of the assay sera positive to the closely related organism B.bovis or to the piroplasm Theileria annulata were also included in the analysis. A total of 133 sera samples was analyzed in duplicate: 28 positive only to B.bigemina, 14 only to B.bovis, 51 and 18 respectively positive and negative to both these organisms, 22 positive only to T. annulata. Horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies were used to detect the presence of antibodies anti-AMA-1. The optical density (OD) was measured at 405 nm.

The B.bigemina AMA-1 antigen was obtained in a good quality and amount (20 µg/ml) and used to set up the best conditions for an ELISA protocol. Significant differences were obtained between sera negative to both B.bigemina and B.bovis (average OD 0.42± 0.11) and samples positive only to B.bigemina (average OD 0.90± 0.13), only to B.bovis (average OD 0.99±0.18) or to both the pathogens (average OD 1.15±0.12). No significant reaction was instead observed in presence of T.annulata positive sera (average OD 0.40±0.13).

Hereby is described the successful method of the in E.coli synthesis of B.bigemina AMA-1 protein and are showed the data of the purified antigen application in a new diagnostic test. The results showed that this protein is suitable to be used as antigen in a diagnostic assay useful for babesiosis diagnosis in cattle, without cross reaction with anti T.annulata antibodies.
Research supported by the Italian Ministry of Health (IZSSI 11/10).

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MICROBIOLOGICAL AND PARASITOLOGICAL SURVEY IN URBAN PIGEONS LIVING IN EXTERNAL HOSPITAL AREAS IN UMBRIA (CENTRAL ITALY)

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The urban pigeon (*Columba livia*) is considered an important reservoir of potentially zoonotic agents, with severe sanitary implications. Pigeons frequently live in colonies in urban and peri-urban centers such as squares, public parks or green areas near hospitals where susceptible subjects or patients may inadvertently be infected by contact with them and their excreta or by inhalation of aerosolized pathogenic organisms (Haag-Wackernagel and Moch, 2004; Andrade-Silva et al., 2010). A microbiological and parasitological survey was conducted in partnership with the USL Umbria1 (local health service) of Perugia in a population of urban pigeons living near a hospital located in the suburbs of Perugia (Umbria, Central Italy).

One hundred pigeons were given a clinical physical examination and were then euthanized and investigated for infectious agents, parasites and fungi. *Coxiella burnetii*, *Chlamydia psittaci* and *Chlamydophila spp*. DNAs were investigated by PCR in 25 fecal pools. Individual caecal contents were analyzed to detect the presence of *Salmonella spp*. and *Campylobacter spp*.. Colonies were identified by biochemical tests and observed under a light microscope after Gram staining; *Campylobacter* isolates were then identified with API-Campy REF20800 and ApiWeb (BioMérieux). Fecal samples of each pigeon were submitted to routine flotation method and to ELISA test (ProSpecT, Oxoid) for the recovering of *Giardia spp*. and *Cryptosporidium spp*. coproantigens. Individual serological samples were tested with Modified Agglutination Test (ToxoScreen, BioMérieux) to detect antibodies against Toxoplasma gondii. Cloacal swabs were carried out on all animals and then cultured into Sabouraud Dextrose Agar and Caffeic Acid Agar to detect yeasts and moulds; the yeasts were subsequently identified by biochemical tests (API ID32C, BioMérieux).

Pigeons did not show any clinical signs. All fecal pools were negative for *C. burnetii* DNA, while one pool was positive for *C. psittaci* DNA and 2 pools were positive for *Chlamydophila spp*.. The results concerning bacteria, parasites and fungi detected by individual samples are shown in Table 1.

Even though all the pigeons were apparently healthy, several pathogens were detected. It's important to emphasize that the finding of *C. psittaci*, *C. jejuni subsp. jejuni* and coli and yeasts including some emerging pathogens might represent a potential hazard for human health, especially when the colony is close to a hospital. Further epidemiological and biomolecular investigations should be carried out in order to implement biological data.

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Table 1: Prevalence of bacteria, parasites and fungi detected in pigeon samples.

BACTERIA			PARASITES			FUNGI		
	N° Positive samples	(%)		N° Positive samples	(%)		N° Positive samples	(%)
***Salmonella* spp.**	0	0%	***Toxoplasma gondii***	8	8%	**Yeasts**	33	33%
***Campylobacter* spp.**	12	12%	***Giardia* spp.**	0	0%	*Candida zeylanoides*	2	2%
						Candida famata	5	5%
-Campylobacter jejuni* subsp. *jejuni	12	12%	***Cryptosporidium* spp.**	0	0%	*Candida incospicua*	1	1%
-Campylobacter jejuni* subsp. *coli	4	4%	**Other endoparasites**	61	61%	*Candida lusitaniae*	3	3%
						Cryptococcus albidus	4	4%
			-*Eimeria* spp.	48	48%	*Rhodotorula mucilaginosa*	3	3%
			-*Capillaria* spp.	29	29%			
			-Tapeworm	8	8%	*Rhodotorula glutinis*	1	1%
			-Roundworm	10	10%	*Zygosaccharomyces* spp.	13	13%
			-Co-infection rate *Eimeria* spp. + *Capillaria* spp.	19	19%	*Trichosporon inkin*	1	1%
			-Co-infection rate *Eimeria* spp. + Roundworm	10	10%	**Moulds**	4	4%
						Penicillium spp.	3	3%
			-Co-infection rate *Capillaria* spp. + Tapeworm	5	5%	*Aspergillus* spp.	1	1%

EVALUATION OF THE PERFORMANCES OF A RAPID ENZYME LINKED IMMUNOSORBENT ASSAY IN THE DETECTION OF ANAPLASMA PHAGOCYTOPHILUM ANTIBODIES IN HORSES

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A simple qualitative in-clinic Enzyme Linked Immunosorbent Assay, the Snap®4Dx (IDEXX Laboratories) test was recently marketed for use in dogs to identify antibodies directed against an Anaplasma phagocytophilum p44 protein. Several studies reported a strong reactivity of such test with sera of horses affected by Equine Granulocytic Anaplasmosis (EGC) even though evidence supporting the interspecies application still remained very limited (Hansen et al., 2010). Aim of the present study was to determine the performances of this rapid test, in the detection of A. phagocytophilum antibodies in serum samples of horses.

The study was carried out on a population of 444 horses consisting on 200 apparently healthy animals(Group A), reared in multiple locations in Central Italy, highly endemic for Tick Borne Diseases (TBD's), and 244 subjects (Group B) with clinical signs or hematologic abnormalities suggestive of TBD's.
Sera obtained from the studied population were tested for A. phagocytophilum IgG antibodies both with the Snap®4Dx kit and an Indirect Fluorescence Antibody Test(IFAT) using commercial antigens.
The level of agreement between the Snap® and IFAT was assessed with Kappa statistics (k) and McNemar's Chi-square test, using IFAT as the comparative test. The relative performances of the Snap® were also evaluated for the different groups (A and B).

N. 45 out of the 444 horses tested positive for IgG antibodies using IFAT, in comparison 40 horses tested positive using the Snap® test. Any significant differences in the proportion of A. phagocytophilum positives between tests ($p > 0.05$) was observed; 39 animals tested positive using both Snap® and IFAT, with an overall agreement of 98.4% and a k value of 0.93 (almost perfect). Snap® testing exhibited high overall performance indexes: Sp (99.75%) as well as PPV (97.5%) were high, whereas there was a slightly lower value of Se (86.67%)(Table).
Among the 45 IFAT positive animals, 18 belonged to the A group and 27 to the B group; conversely, among the 40 horses tested Snap® positive, 15 subjects belonged to the A and 25 to the B groups respectively. No statistically significant differences in seropositive rates obtained using Snap® and IFAT testing were observed between the two groups ($p > 0.05$). The level of agreement between the two tests was high and almost identical in A and B groups as well as their Se and Sp values.

The present study indicates that the Snap® test possesses high specificity and sensitivity in the detection of A. phagocytophilum antibodies in horses and could represent a valid screening method for epidemiological survey of EGC.

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Burgdorferi infection in horses. J Vet Diagn Invest 2011; 20: 321-324.

Table - Cross-tabulation of Snap® 4Dx test results and performance indices in different clinical groups (A and B), using IFAT as a comparative test. Confidence Interval (CI); Kappa value (K); Sensitivity (Se); Specificity (Sp); Positive Predictive Value (PPV); Negative Predictive Value (NPV)

Snap® 4Dx		IFAT		McNemar's chi-square test (P-value)	K	Se (95% IC)	Sp (95% IC)	PPV (95% IC)	NPV (95% IC)
		Pos	Neg						
Total animals	Pos	39	1	0.059	0.93	86.67% (71.85-93.65%)	99.75% (97.95-99.96%)	97.50% (95.60-98.70%)	98.51% (95.40-99.30%)
	Neg	6	398						
A group	Pos	15	0	0.083	0.90	83.33% (80.22-91.54%)	100%	100%	98.38% (93.4-99.71%)
	Neg	3	182						
B group	Pos	24	1	0.31	0.96	88.89% (67.56-96.16%)	99.54 (96.32-99.93%)	96% (93.45-98.65%)	98.63% (96.43-99.86%)
	Neg	3	216						

TICK BORNE PATHOGENS IN SICILIAN EQUIDS

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Piroplasmid protozoa Theileria equi and Babesia caballi and zoonotic rickettsial bacterium Anaplasma phagocytophilum are important agents of equine vector-borne diseases (EVBD). Equine piroplasmosis can cause acute, sub-acute or chronic diseases. It has a worldwide distribution and it is endemic in most tropical, subtropical and some temperate zones. A. phagocytophilum, the causative agent of granulocytic ehrlichiosis, affects several species of wild and domesticated mammals, including horses and donkeys.
This study was aimed to investigate the EVBD prevalence in Sicilian equids using both serological and molecular diagnostic methods.

Serum samples from 145 equids were analyzed for the presence of antibodies against B. caballi, T. equi and A. phagocytophilum using the Immunofluorescence Antibody Test (IFAT). Eighty blood samples were also subjected to DNA extraction to carry out PCR assays in order to detect pathogen DNA.

A total of 12 (prevalence of 8,3%) and 56 (38,6%) equids were found to be serologically positive for B. caballi and T. equi, respectively, while 7 (4.8%) equids were serologically positive for A. phagocytophilum.
No animals were positive for B. caballi and A. phagocytophilum by PCR assay, while 38 equids (47.5%) resulted to be positive for T. equi.

This study showed that EVBDs are indeed present in Sicily. T. equi and B. caballi seem to be the most important EVBD pathogens. According to several results reported by other Italian researchers, Italy represents a high risk area both for the vector presence and geographical and climatic conditions. For all these reasons symptoms attributable to EVBD should be promptly reported to veterinary authorities.

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EPIDEMIOLOGY OF NOSEMA CERANAE AND NOSEMA APIS IN HONEY BEES FROM AOSTA VALLEY, ITALY.

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Nosema apis and N. ceranae are microsporidian parasites that affect adult honey bees Apis mellifera. N. ceranae has spread rapidly across the world and it is considered to be one of the risk factors related to "Colony Collapse Disorder" even if its pathogenic role has never been fully clarified (Higes et al., 2009). This parasite causes a reduction of colony's productions, depopulation of affected families, and it is present in Europe since at least 1993 (Ferroglio et al., 2013). The goal of this study was to assess presence and prevalence of the parasites within the Aosta Valley Region (Italy), and to investigate management and environmental factors to reduce the risk of infection.

The research project involved 15 apiaries from September 2012 to April 2013. Five hives from each apiary were sampled twice in autumn and once in spring (n=75 hives sampled). From each hive a pool of 20 adult bees was analyzed for the presence of spores (Burker's chamber) and for N. ceranae and N. apis DNA by qualitative PCR (Ferroglio et al., 2013) and quantitative real-time PCR using an in-house protocol with Taqman probes.

N. apis was never detected in any of the apiaries, while N. ceranae PCR prevalence was 20% in the first autumn sampling , and increased to 37% in spring. Statistical and spatial analysis evidenced that high solar radiation and the presence of agricultural activities in a 3 km2 area around the hives are relevant environmental parameters for preventing N. ceranae infection. Bees of the local Carniolan subspecies (Apis mellifera carnica) were statistically more resistant to infection than Italian bees Apis mellifera ligustica (χ^2=9.75, p=0.0018).

In the apiaries of Aosta Valley Region where N. apis seems to have disappeared, N. ceranae is widely present. Its epidemiology seems to be deeply related to environmental and management factors. Our data confirm for N. ceranae, what Malone et al.(1995) reported for N. apis, that Carniolan bees are less susceptible to infection than the Italian subspecies. Further studies on genetic selection of resistant breeds, and on improved managing practices are needed.

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SEROSURVEY FOR SCHMALLENBERG VIRUS IN ALPINE WILD UNGULATES

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Schmallenberg virus (SBV), a novel member of the family Bunyaviridae, was detected in cattle in North-Western Europe in 2011. SBV infections have been reported as the cause of congenital malformations and stillbirths in cattle, sheep, and goats. The first case in Italy was reported during mid-February 2012 in cattle in the Veneto region. Seropositivities in wild animals were assessed in various countries, but the occurrence of SBV infection have not yet been described in any wild species in the Alpine region.

Therefore, we conducted a serosurvey to assess the presence of SBV-specific antibodies in free-ranging alpine ruminants in an area located between Stelvio and Adamello National Parks characterized by a high red deer density (16 animals/100 hectares). Serum samples from chamois (23) and red deer (352) hunted from 2007 to 2013 were tested by ELISA kit (ID Screen® Schmallenberg Virus Competition, IDvet) and confirmed by the virus-neutralization test (VNT), using Vero cells and SBV strain BH80/11-4 (RBV 1099-FLI), (FLI institute,Germany).

All of the serum samples collected through September 2012 tested negative, whereas a single chamois serum and 21 red deer sera taken during the 2012-2013 hunting season tested positive.

Vector transmission (Culicoides spp) of SBV has been demonstrated in different European countries. Midges could play an active role in the spread of the virus, and their implication in the epidemiological cycle could explain the fast and wide spread of SBV. The diffusion of SBV in the study area could have occurred between the January and December 2012. Biting midges of the genus Culicoides are not usually active in the Alpine region from week 47–49 to week 12–14, depending on weather conditions. SBV infections could be occurring in the warmer months when Culicoides can reach high altitudes, including alpine summer pastures.
SBV is not an OIE World Animal Health Information System notifiable disease, but the economic impact of the disease at farm level should be considered, particularly in the rural areas of the Alps. Conceivably, domestic ungulates grazing on common pastures with red deer and chamois during the summer could confer a role for wildlife in the epidemiology of SBV in the alpine environment.
To our knowledge, this is the first report of SBV infection in Alpine wildlife in Italy. Thus, there should be the targeted surveillance of wild ruminants to assess the epidemiological role of wildlife.

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MOLECULAR CHANGES ASSOCIATED WITH ADAPTATION OF AVIAN INFLUENZA VIRUSES IN EMBRYONATED CHICKEN EGGS AND CELL CULTURES.

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The aim of this study was to evaluate potential adaptive mutations in hemagglutinin (HA), neuraminidase (NA) and non-structural protein (NS1) encoding regions of different Avian Influenza viruses (AIVs) subtypes following serial cultivation, either in chicken embryos (CE) or in established cell lines.

Selected viruses subtypes were represented by: A/mallard/Italy/3401/05 LPAI H5N1, H5N2 HPAI A/chicken/Italy/8/A98, A/turkey/Italy/4580/99 HPAI H7N1, H7N3 LPAI A/turkey/Italy/2962/V03, H9N2 LPAI A/turkey/Wisconsin/66. Three types of established cell lines: NSK (Newborn Swine Kidney), MDCK (Madin-Darby Canine Kidney), UMNSAH/DF1 (Chicken embryo fibroblasts) were used for AIV primary isolation from target tissue and serial amplification. The harvested supernatants of infected samples were used for RNA extraction and subjected to reverse transcription for cDNA synthesis and PCR reactions. PCR products were purified and used for sequencing reactions.

Hemagglutinin (HA), neuraminidase (NA) and non-structural protein (NS1) encoding regions were sequenced and compared to the GenBank sequences.The results showed different point mutations in HA and NA encoding regions in all AIV subtypes grown either in CE or in cell cultures. The genetic analysis performed on different influenza virus subtypes grown either in chicken embryos or in cell lines have shown that amino acid substitutions detected do not induce alterations of the genetic characteristics and biological properties of viruses.

Several studies demonstrated that serial propagation of influenza virus in CE can be responsible for surface glycoproteins amino acid sequence substitutions, resulting in subtypes that differ antigenically from the original wild type (1, 4, 5).On the contrary, viruses propagated in mammalian-derived cell cultures don’t undergo to mutations and maintain the protein sequence of the original virus (1, 2).The critical role of mutations within the avian virus genome highlighted the importance of understanding the molecular basis related to the interaction events between host and AIVs. The genetic analysis performed on different influenza virus subtypes grown either in chicken embryos or in cell lines have shown that amino acid substitutions detected in HA, NA and NS1 regions did not result in a virulence modification as observed in the pathogenicity tests (3).Cell cultures can represent a valid biological system alternative to chicken embryos for the in vitro amplification of Avian Influenza viruses.

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SALMONELLA SEROTYPES IN WILD BOARS (SUS SCROFA) HUNTED IN NORTHERN ITALY

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Salmonella species (spp.) are zoonotic enteric bacteria able to infect humans, livestock and wildlife. Salmonella spp. have been sporadically isolated from the intestinal content of wild birds and mammals including white-tailed deer, rabbits and wild boars (Sus scrofa). However, little is known about the prevalence and the presence of the different serovars in wildlife.

Over six hunting seasons, the intestinal contents of 2,729 boars hunted in northern Italy were sampled and cultured. Salmonella spp. was isolated following the methods reported in "Annex D ISO 6579:2002", mandatory in the implementation of Salmonella monitoring and control plan for primary productions. All presumptive Salmonella spp. isolates were confirmed using biochemical tests.

Salmonella spp. were isolated from 629 boars (21.06%). The isolates belonged to 48 different serovars classified into three different subspecies of S. enterica: S. enterica subsp. enterica (n=489 strains, 77.75%, 38 serovars), S. enterica subsp. diarizonae (n=73, 11.60%, 7 serovars) and S. enterica subsp. houtenae (n=67, 10.65%, 4 serovars). Thirty-eight serovars of S. enterica subsp. enterica were found, including the human pathogens S.Typhimurium and S. Enteritidis, as well as S. Napoli and S. Enterica 4,5,12:i:-), a monophasic variant of S. Typhimurium, some of which are are emerging serovars both in humans and animals. In addition, the identification of serovars belonging to S.enterica subsp. diarizonae and S. enterica subsp. houtenae prove how wild boars may act as healthy carriers of a wide range of Salmonella serotypes.

Considering the widespread occurrence of wild boars in Europe and the feeding behaviour (omnivorous scavengers), this specie may be considered a good indicator for environmental presence of Salmonella spp. Therefore, the epidemiological role of this species in relation to salmonellosis might be relevant and should be further investigated.

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BIOFILM FORMATION IN MULTIDRUG-RESISTANT CANINE PYODERMA ISOLATES OF STAPHYLOCOCCUS PSEUDINTERMEDIUS

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The control of Staphylococcus pseudintermedius infection is often difficult due to the expanded multi-drug resistant (MDR) S. pseudintermedius (MDRSP) strains and methicillin resistant S. pseudintermedius (MRSP) strains. Casagrande Proietti et al. (2012) reported that all tested S. pseudintermedius isolates from canine pyoderma were multi-drug resistant and MRSP rate (41.4%) was higher than elsewhere reported. It is known that antibiotic resistance in staphylococcal species is often associated with biofilm, that consists of layers of cell clusters embedded in a matrix of extracellular polysaccharide, which adheres to biological or inert surfaces (Oliveira et al., 2006).The present study aimed to investigate the differences in the prevalence of the resistance to 15 diverse antibiotic drugs and the ability to biofilm formation in S.pseudintermedius canine pyoderma isolates.

A collection of 36 S.pseudintermedius isolates from canine pyoderma were included in this study. Susceptibility to a panel of 14 antimicrobial agents was determined by the disk diffusion method in Mueller-Hinton agar. The ability of the isolates to form biofilm was determined by the electron microscopy (TEM) and by the tissue culture plates (TPC)

All isolates showed biofilm production on microtitre plates, except for two isolates which gave conflicting results.In particular sixteen out thirty-four isolates were classified as weakly, seven as moderately and eleven as strongly adherent, according to Stepanovic et al. (2000). Twenty-seven out thirty-four isolates were resistant to nine or more antibiotics. At TEM examination, in every sample, biofilm appeared as a well contrasted, variably extending fibrillar tree. In the examined samples the fibrillar structure was present weakly, moderate and strong.

In this study we have confirmed the concordance between the ultrastructural aspect of some strains with the results on the microtitre plate method. Interestingly all isolates showed biofilm production and MDR phenotype. All isolates exhibited simultaneous resistance to at least six antibiotics, more than one in three (38.9%) of the isolates showed resistance to at least eleven antibiotics, and two isolates were resistant to all the fifteen antibiotics.These results suggest a relationship between multidrug resistance of clinical S. pseudintermedius isolates and biofilm production. The lowered susceptibility appears to be attributed to insufficient penetration of antibiotics into biofilm and the reduced growth rate of bacteria embedded in biofilm. Very little is known about genetic control of biofilm production in S. pseudintermedius. At this time we are investigating the presence of icaA and icaD genes mediating the synthesis of the capsular polysaccharide in S. pseudintermedius and our preliminary results demonstrated their presence in isolates from canine pyoderma.

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EVIDENCE OF BPV-1 MIXED POPULATIONS IN BLOOD OF HEALTHY HORSES

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Papillomaviruses (PVs) are small, circular, non-enveloped, double stranded DNA viruses that infect mucosal and cutaneous tissues of many vertebrates, inducing papillomas of squamous epithelia. PVs infect a large range of hosts in nature, yet each virus species is generally species-specific and even in experimental conditions do not infect any other host than their natural one (1). Only the delta (2) BPV (Bovine Papillomavirus)-1 and, more rarely, BPV-2 infect horses, donkeys and mules (3, 4,5) causing sarcoids, defined as a locally aggressive fibroblastic benign tumors of the skin (6).
The genome is divided into three canonical regions: a long control region, containing the regulatory element, a region containing the late genes encoding the capsid proteins, and a region containing the early genes encoding non-structural proteins. The E5 oncogene encodes for the major viral oncoprotein of BPV. Since BPV-1 E5 variants are known to exist in sarcoids of donkeys (7) and horses (8), we investigated whether this genetic variability might be also found in BPV-1 PBMC associated of sub-clinically infected horses.

With this aim we amplified the E5 gene of 22 BPV-1 strains from diseased (12) and subclinically infected horses (10).
All chromatograms of the amplified products were visually inspected to detect the presence of sequences with ambiguous or polymorphic bases, suggesting the presence of mixed viral populations in 5 subclinically BPV-1 infected horses. For this reason the amplification products, where evidence of mixed population was detected, were cloned into the pDriveCloning vector (Qiagen, Hilden, Germany).

This further analysis lead us to demonstrate that multiple virus variants can be present in the blood of some subclinically infected horses, with alternative bases corresponding to either synonimous and non-synonymous codons in the E5 oncogene sequences.

The mutations in the E5 oncogene of BPV-1 affecting apparently healthy horses confirm the sequence variability of this gene, but do not allow to prove the existence of an equine-associated BPV-1 E5 variant, as stated by (9). Instead, our results give further support to (10) that proposed the existence of “equine adapted” BPV-1 strains. As risk factors for pathology development remain equivocal, the possibility to an early diagnosis based on the detection of BPV-1 E5 sarcoid associated nucleotide mutations might be useful to improve the prevention of the disease.

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SEROLOGICAL AND MOLECULAR SURVEY OF AUJESZKY'S DISEASE IN WILD BOAR (SUS SCROFA) IN NORTHERN ITALY

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Aujeszky's disease (AD) is a highly contagious, economically significant disease caused by Pseudorabies virus (PrV), principally affecting wild and domestic pigs. AD can result in trade restrictions from regions where it is endemically present. As a consequence, eradication programs in swine and surveillance programs on wild boar are ongoing. Although reports of PrV transmission from wild boars to domestic pigs are sporadic, success in disease eradication programs in the livestock could be influenced by wildlife reservoirs. Despite wild boar seems to serve as a persistent reservoir for PrV, there is still a lack of data describing the AD epidemiology in free ranging wild boars population. Therefore, the aim of this study was to determinate the temporal dynamic of AD infection in wild boars hunted in an area of North Italy.

A total of 2847 sera samples of hunted free-living wild boars were collected from 2006 to 2013 and then tested by a competitive ELISA for AD-gE. Additionally, from 2011 to 2013, 878 wild boar amygdales were collected and analysed by real time-PCR in order to investigate the presence of PrV in a latent form. In the last decades, an overall increasing of the wild boar populations was registered in the 8 hunting districts here considered. They are located in the footstep mountain in Northern Italy (Province of Brescia, Lombardy), where swine herds are not present.

One hundred and nineteen wild boars (4.2%) were seropositive and real time PCR resulted positive in 8 cases (1.22%). Even if the infection is present since 2006, seroprevalences higher than 9.4% have never been detected. PrV is present and persistently circulating in an isolated population, located in an area where swine herds are not present.

The obtained data and the absence of pigs farms in the study area could indicates that wild boars can be considered maintenance host for PrV regardless of the presence of pig farms.

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IDENTIFICATION OF BACTERIA FROM CARETTA CARETTA TURTLES STRANDED IN SICILIAN COASTS

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In the last six months a high number of stranded turtles has been observed in Sicilian coast. Marine turtles have been proposed as sentinel species useful as environmental health indicators for coastal marine habitats (Aguirre and Lutz, 2004; Owens et al., 2005). Ecological and physiological characteristics make them very reliable bio-indicators (Foti et al., 2009). C. caretta is included in the Red List of the world conservation union and is highly threatened. The aim of this study was to report the microbiological analyses of twentynine stranded marine turtles in Sicilian coasts in the first six months of 2013.

A total of 29 turtles were reported as stranded between January and July 2013. Twenty of them were recovered and subjected to necroscopical examination. All the examined sea turtles were measured and weighted and the species identification was carried out. Microbiological exams were carried out from the organs collected from every turtle using different culture media. The Gram-staining, catalase and oxidase activity tests were performed on all isolated strains. Moreover further characterization was carried out using biochemical tests.

All the turtles were identified as Caretta caretta. Anatomical and pathological exams revealed the presence of hooks and plastic debris in various portions of the alimentary tract in many specimens. In other turtles, lesions in the carapaces or plastrons, probably due to collision with boats, were found. Moreover, lesions were found also in the limbs and in the head. Microbiological exams carried out from organs taken from every turtle. Almost half of the examined turtles revealed in their organs the presence of Gram negative bacteria (E. coli, Klebsiella spp., Citrobacter spp., Shewanella spp. and Salmonella).

These are preliminary results. The following step will consist in the antibiogram assessment in order to evaluate possible differences in antibiotic resistance between marine and terrestrial animal isolated microorganisms. The other goal will be Salmonella characterization.

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Enteric bacterial pathogens in growing pigs from farrow-to-finish herds.

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Pig salmonellosis, yersiniosis, porcine proliferative enteropathy (PPE), and swine dysentery (SD) are considered important diseases in grower-finisher pigs, being able to cause mortality and suboptimal performance. The aim of this study was to evaluate the presence of *Salmonella spp*, *Yersinia enterocolitica*, *Lawsonia intracellularis*, *Brachyspira pilosicoli* and *Brachyspira hyodysentieriae* in fecal samples of asymptomatic growing pigs.

Faecal samples of growing pigs (30-60 kg) were taken from 19 farrow-to-finish farms. From each farm, pooled samples of fresh faeces were taken from 10 different barns containing 25-40 growing pigs. Detection of *Salmonella* spp has been performed according to the ISO 6579:2002 /Amd 1:2007 method, the detection of *Y.enterocolitica* has been performed according to the ISO 10273:2003 method, detection of *L.intracellularis* has been performed by using Real Time PCR, and detection of *B.hyodysentieriae* and *B.pilosicoli* has been detected by bacterial colture and PCR Real Time according to the method reported elsewhere (Willems and Reiner, 2010).

Thirty-four out 190 samples (19%) were positive to *Salmonella* spp. Of those, 22 samples were positive for *S*.Typhimurium monofasic, 10 were positive for *S*.Derby, 1 for *S*.Infantis and 1 for *S*.Mbandaka. Eighty-one out 190 samples (46%) were positive for *Y.enterocolitica*, 57 out 190 samples (32%) were positive for *L.intracellularis*, 19 out 190 samples (10%) were positive for *B.pilosicoli* (10,7%), and none out 190 sample was positive for *B.hyodysentieriae*.

These data confirm that bacterial enteric pathogens are common in asymptomatic growing pigs and highlight the need for an active and targeted investigation in farrow-to-finish herds.

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PRELIMINARY RESULTS FOR THE IMPLEMENTATION OF PROTEINS PRODUCTION IN LEPIDOPTERA LARVAE (TRICHOPLUSIA NI), EXPRESSED BY BACULOVIRUSES.

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A large number of proteins has been successfully expressed in insect cell lines infected with baculovirus (1). In IZSLER the expression of foreign proteins using Sf9 insect cell line infected with recombinant baculoviruses is being active since several years, however this system of production has a potential disadvantage in the cost associated with large-scale tissue culture operations.
Compared to production in cell culture, insect larvae can produce proteins at reduced cost (2). The main aim of this study is to establish a laboratory stable colony of larvae Trichoplusia ni to be employed as hosts for expression of foreign proteins by infection with recombinant baculoviruses.

A colony of T. ni was maintained at the constant temperature of 26°C, relative humidity of 65% and photoperiod (16:8 light/dark). Two different diets were compared in supporting larvae growth.
The Diet 1 was based on Shorey study (1965) and it was composed of soaked beans and brewer's yeast, whereas the Diet 2 was the "Modified form MCmorran Grisdale commercial Diet".
For the proteins production fifth-instar larvae were infected individually by injection of 0.1 ml of high titer recombinant baculoviruses stock, expressing the non-structural protein 3 (NS3) of BVDV or the capsid protein (ORF2) of HEV. Infected larvae were kept in incubator at the condition described above and collected after 72h. Larvae were then immediately frozen and kept at −80 °C until processed.

The Diet 1, in respect to the Diet 2, seemed to allow fast development of each stage, shortening of about 28 h the entire development time needed by larvae to reach adult stage. However, in the experiment carried out to test the substrate preference, larvae of the first stage moved towards diet 2 and rejected the diet 1 suddenly after eggs hatching, showing that the alimentary preference of the colony could be important in the establishment of a laboratory stable colony.

The yield and reactivity of the two proteins were preliminary evaluated with specific Monoclonal Antibodies (MAbs) and positive serum samples in ELISA assays. Both BVDV-NS3 and HEV-ORF2 were produced at significantly higher levels than those obtained by infecting insect cell cultures while maintaining the antigenic properties.

Results encourage the exploitation of this production system both for internal use and as a service.
Next steps will investigate different inoculation routes (baculovirus injection compared with oral administration with the diet), different payloads and best protein extraction procedures.

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF CANINE PYODERMA ISOLATES OF S.PSEUDINTERMEDIUS FOR BIOFILM FORMATION

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Biofilm-forming ability is increasingly being recognized as an important virulence factor in several staphylococcal species, causing chronic infections which are difficult to treat. The principal component of biofilm is a polysaccharide intercellular adhesion (PIA), codified by the ica operon found on the bacterial chromosome, that includes a regulating element of four genes(A,B,C and D).The coexpression of icaA with icaD leads to a significant increase in activity and is related to phenotypic expression of the biofilm (Arciola,2001).The aim of this study was carried out to determine the biofilm producing ability as well as the presence of the icaA and icaD genes in canine pyoderma isolates of S.pseudintermedius

A collection of 60 S.pseudintermedius isolates were included in this study. The biofilm producing ability was determined by cultivation on Congo Red Agar (CRA).The production of black colonies biofilm producing strains was used to differentiate them from non-biofilm producing S.pseudintermedius strains which appear red on CRA. For PCR analyses primers used and PCR conditions were described in Table 1.

Among the 60 S.pseudintermedius isolates tested, 54 strains (90%) were found to produce typical black colonies on CRA. 55 (91,6 %) strains were found to possess the icaA e icaD genes. Among the five PCR-negative strains two isolates produced black colonies and three produced red colonies on CRA. Three out fifty-five icaA/icaD positive strains showed red colonies on CRA.

The data reported in our study indicate the icaA and icaD genes in a high percentage of clinical isolates and its association with the strains' ability to produce biofilm, suggest a role of these genes in the pathogenetic mechanism of canine pyoderma. Our results showed that two isolates produced black colonies on CRA although not possessing icaA and icaD genes. These two types show that as well as PIA, other protheins and carbohydrates might be involved in the phenotypic biofilm production (Chokr 2006). Three of fifty-five icaA/icaD positive strains showed red colonies on CRA: it could depend from the environmental factors that strongly influenced ica expression. Moreover, ica operon expression can be turned on and off by the insertion and excision of the insertion sequence IS256, which was found in S.pseudintermedius also (Casagrande 2011).Considering the role of the biofilm in the evasion of the immunological defenses and the resistance to antibiotic therapy, our findings have high significance.

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Table 1. Primer used in this study, and PCR conditions.

Gene	Primer Name	Sequence (5'-3')	Amplicon Length	PCR Conditions
icaA	icaA-Fw	ACTGTTTCGGGGACAAGCAT	134 bp	94°C x 3min, 35 x (94°Cx 15 s, 60°C x 20 s, 72°Cx 20 s) 4°C 8
	icaA-Rev	ATTGAGGCTGTAGGGCGTTG		
icaD	IcaD-Fw	CGTTAATGCCTTCTTTCTTATTGCG	166 bp	94°C x 3min, 35 x (94°Cx 15 s, 56°C x 20 s, 72°Cx 20 s) 4°C 8
	IcaD-Rev	ATTAGCGCACATTCGGTGTT		

SEROLOGICAL SURVEILLANCE OF LEPTOSPIROSIS IN ITALY: TWO YEAR PERIOD NATIONAL DATA (2010-2011)

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Leptospirosis is a notifiable zoonosis, ruled by the "Regolamento di Polizia Veterinaria" (DPR 320/54). Nowadays, leptospirosis is a re-emerging widespread infectious disease probably worldwide underestimated. The National Reference Centre for Leptospirosis (NRCL) evaluated the distribution of such important zoonosis in Italy with the cooperation of all the other Istituti Zooprofilattici Sperimentali (IIZZSS). Serological data obtained during the two year period 2010-2011 by each laboratory were collected by the NRCL.

The microscopic agglutination test (MAT) is the standard serological test, as described by the OIE guidelines. The panel of antigens is composed by 8 serovars which are representative of the serogroups known to exist in the Italian area. Sera samples showing titers higher than the MAT cut-off of 1:100 against one or more serovars were considered positive. Serological data were analysed and grouped on the basis of low (≥ 1/100), medium (= 1/400) and high titers (≥ 1/800) detected against only one serovar.

During the two year period, 43935 sera samples collected from different animal species were analyzed. Bovine (46.86%), swine (27.46%), ovine and goat (7.35%), dog (6.89%) and wild boar (4.52%) samples were delivered to the Laboratories more frequently than the equine and other domestic species sera. 6279 samples (14.3%) resulted positive at MAT with titers higher than (≥) 1/100.
Data analysis showed that the most common serogroups in Italy are: Australis in dog, wild boar, horse, hare, swine, fox and rodents; Sejroe in cattle, sheep, goat and buffalo; Icterohaemorrhagiae in dog, goat and fox; Pomona in swine and wild species; Grippotyphosa in hare.

The purpose of this work was to update the knowledge about the prevalence and the distribution of leptospirosis in Italy throughout the collection and the elaboration of serological data given by the IIZZSS and concerning the 2010-2011 serological activity.
The creation of a network able to collect all national laboratory data could be useful for the surveillance of leptospirosis in Italy. Furthermore, the exchange of these information between the Italian and International Health Institutions should be supported.

OIE (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.9: 251-264.

HEPATITIS A VIRUS (HAV) MOLECULAR CHARACTERIZATION: CORRELATION BETWEEN CLINICAL CASES AND FOODSTUFFS

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In Italy, a specific sentinel surveillance system for acute viral hepatitis (SEIEVA) allows the prompt evaluation of incidence and trends of the disease and insight into the risk factors.
From January to May 2013 a total of 352 cases of hepatitis A were reported, corresponding to a 70% increase in HAV notifications compared to the same period in 2012. The highest increase in the number of cases was observed in the Northern regions that accounted for 55% of the total cases. In Trentino Alto Adige 38 HAV cases were identified. On May 2013, Germany, the Netherlands and Poland reported 15 cases of HAV infection associated with a ski holiday in Trentino Alto Adige. The sequencing of the VP1/2A genomic region of the German and Dutch isolates showed 100% similarity each other and with one of the Trentino Alto Adige isolates, corresponding to HAV1A strain. The epidemic curve suggested a common source.
Preliminary epidemiological investigations for the identification of risk factors focused on consumption of mixed frozen berries. As a consequence Italy notified through the RASFF (Rapid Alert System for Food and Feed) the HAV findings in frozen berries. Moreover, after the positive results on the sampled berries from different regions the Ministry of Health started the tracing back of the food item. The investigation identified a dealer that received consignments of berries from different countries (Italy, Poland, Bulgaria, Canada and Serbia). Following the RASFF notification, regions recalled the positive lots and advised the population regarding the use of the leftover frozen mixed berries.

In the period May-June 2013, 92 food samples were tested for Official HAV Controls at the IZSLER in Brescia. Other 43 samples were analyzed for Food Business Operators self check; 10 stool samples for hepatitis A-affected patients were sent by three different hospitals. The 85,5% of foodstuffs (Official and self check) was constituted by frozen berry fruits, while the remaining 14,5% were ready to eat salads and fruit. Analyses were performed according to the ISO_TS 15216-2. Virus genotyping for positives was performed in the VP1/2A region of the viral genome.

Three (3) berries and 5 salads resulted contaminated, while stool samples confirmed as positive. All of the self check specimens were negative. HAV detected in one raspberry sample was 100% similar to the HAV in stools, and genotyped as HAV1A.

Preliminary analysis of the case interviews on possible risk factors identified consumption of frozen mixed berries. This assumption was supported by the detection of HAV virus in frozen mixed berries. The surveillance on these food items together with other vegetables potentially carrier of the HAV has been intensified, to provide a picture of the distribution of the contaminated items and the risk of exposure through these foods.

Outbreak of hepatitis A virus infection in residents and travellers to Italy. Joint ECDC-EFSA rapid outbreak assessment. Stockholm: ECDC; 2013.

EFFICACY OF ATTENUATED SALMONELLA TYPHIMURIUM ?ZNUABC AGAINST SALMONELLOSIS IN PIGS

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Pigs play an important role in transmission of salmonellosis to humans. Meat contamination with Salmonella spp is considered by European community an important risk factor for human salmonellosis, recently, 26.9% of human cases were attributed to the consumption of pork products in Europe. The use of antibiotics for controlling or preventing Salmonella is not regarded as an option because Salmonella spp. is an intracellular pathogen and there is an high risk of resistance development. Vaccination may represent an attractive and promising alternative tool to reduce salmonellosis in the swine population and limit the prevalence of healthy carriers at abattoir. In a recent study, we demonstrated that S. Typhimurium lacking the ZnuABC transporter (S. Typhimurium ΔznuABC) is a promising candidate live vaccine in different mouse models of S. Typhimurium infection. Now, we test safety and immunogenicity of the vaccine in pigs.

Four groups of pigs of 80 – 100 days of age were enrolled in the study. Pigs were divided into 4 groups. Groups A, B, C were of 6 animals; group D was of 8 animals. Groups A and B were vaccinated orally with a dose of 5x108 or 5x107 CFU of S. Typhimurium ΔznuABC, respectively. Group C was intramuscularly vaccinated with 2x109 CFU of a formalin inactivated S. Typhimurium ATCC14028 adsorbed on aluminium hydro-oxide and boosted 14 days after with the same dose. Saline solution was administrated at Group D, used as naïve control group. Thirty-four days after vaccination, all groups of animals were challenged orally with 4x108 CFU of fully virulent S. Typhimurium.

Clinical signs of salmonellosis, faecal shedding and organ colonization were used as parameters to assess the protection induced by vaccination. After the challenge, pigs vaccinated with the attenuated S. Typhimurium ΔznuABC strain did not display clinical signs of salmonellosis (fever or diarrhoea). Moreover, they shed virulent S. Typhimurium ATCC 14028 significantly less and their organs result less colonized compared to the control groups.

The results here reported show that the oral administration of the attenuated S. Typhimurium ΔznuABC strain, following by a challenge infection with a virulent S. Typhimurium strain, reduces the clinical signs, organ colonization and shedding of the virulent strain. These results are in agreement with our previous studies in the mouse models of salmonellosis. Coupled with our results demonstrating the safety and immunogenicity of S. Typhimurium ΔznuABC, these findings extend the validity of this candidate attenuated strain as a useful mucosal vaccine in pigs

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AN IMMUNOPEROXIDASE MONOLAYER ASSAY (IPMA) FOR THE DETECTION OF ANTIBODIES TO AFRICAN SWINE FEVER (ASF)

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The aim of this study was to optimize an Immunoperoxidase Monolayer Assay (IPMA) as an alternative to the most common tests (1-3) used to detect antibodies against African Swine Fever (ASF).

To optimize the IPMA, different preliminary experimental tests were conducted using both positive and negative control sera to determine: 1) the best concentration of the VERO cell line; 3) the optimal virus titre; 3) the working dilution of the Protein A HRPO-conjugate; 4) the time of exposure to the chromogenic substrate. The cells revealing an intense red cytoplasmic staining were considered infected.
The IPMA was performed using a set of sera collected from 29 5-week-old pigs in 3 distinct animal experiments conducted by inoculating 3 ASF virus isolates with a different virulence level. The serum samples were collected at 0, 3, 7, 10, 14, 21, 25, 29 and 36 days post-infection (DPI) and were tested by both IPMA and a commercial ELISA test (Ingezim PPA Compac, INGENASA, ES). All tests were conducted in duplicate and repeated 3 times in separate sessions.

Serum samples were tested for ASF antibodies both by IPMA and by a commercial ELISA. Eighty samples reacted positively with IPMA, whereas 26 of them were positive with the ELISA. Particularly, IPMA detected ASF antibodies from 7 to 21 DPI, while ELISA from 10 to 25 DPI.

IPMA resulted more sensitive than ELISA test (2,3) detecting ASF antibodies in earlier infection. Furthermore, IPMA showed unambiguous results in absence of non-specific reactions, therefore it could represent a promising tool as serological confirmatory test, in alternative to the conventional test (1-3).

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EARLY IMMUNE RESPONSES TO TYPE I PRRS VIRUS STRAINS

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To define immunological markers of PRRS virus (PRRSV) pathogenicity

The study was carried out in the framework of two experimental infections of PRRS-naïve pigs with PRRSV strains BS114 / 2000 (group 1) and BSAL / 2011 (group 2). Serum samples were collected weekly until post infection day (PID) 35 to evaluate innate and adaptive immune responses. On PID 56, Peripheral Blood Mononuclear Cells (PBMC) and palatine tonsil cells were cultured and treated as previously described (Razzuoli et al.,2011). The release of PRRSV-specific and non-specific IgA and IgG was measured by Ig isotype-specific ELISAs.

Viremia: PRRSV-infected animals were PCR-positive on PID 7; interestingly, all BS114–infected animals turned negative (with one exception) on PID14, whereas sera of group 2 pigs were positive until PID 21 (3 out of 6), with a nice correlation between fever and viremia.

Interferons: In group 1, little if any in vivo IFN-α response was detected in serum, whereas IFN-γ showed a moderate peak on PID 7. The animals of group 2 showed instead a moderate IFN-α response in serum on PID 3, 7 and 14, and no IFN-γ response. In both groups, no PRRSV-specific IFN-γ response was shown in vitro.

Antibodies: all infected pigs showed specific antibodies by PID 14; in group 1 pigs, PRRSV-specific IgG were detected earlier and at higher titres compared with group 2 pigs. The two strains also showed different responses to non-structural proteins (NSPs); in particular, the response to NSP1β showed different intensity and time-course.

In vitro antibody production. PBMC and tonsil cells of PRRSV-infected pigs did not release PRRSV-specific antibody in vitro. Tonsil cells from pigs infected with the attenuated strain were significantly ($P<0.05$) stimulated to release IgA after treatment with PRRSV+IFN-alpha at 1 or 100 U/ml, as opposed to animals infected with the non-attenuated PRRSV strain.

Early control of PRRSV infection was associated to an innate IFN-γ response to the BS114 strain, which may have contributed to the subsequent decay of viremia. On the other hand , the lack of a type II IFN response could be correlated with the much longer viremia in group 2 animals. Therefore, the active suppression of an early, innate, IFN-γ response in pigs might be a crucial pathogenicity factor of several genotype I PRRSV strains. The different properties of the two PRRSV strains could be partly explained in terms of amino acid changes in NSP1-β, previously recognized as an important modulator of Type I (α/β) IFN responses (Sun et al., 2012). In this respect, BS114/200 and BSAL/2011 showed with respect to the Lelystad reference strain a homology of 98% and 73% in the NSP1-β gene, respectively. This could account for the different IFN-α responses in vivo after infection and the different outcome of the in vitro IFN-α treatments.

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WHOLE-GENOMIC ANALYSIS OF PANDEMIC H1N1 VIRUS IN ITALIAN PIG FARMS

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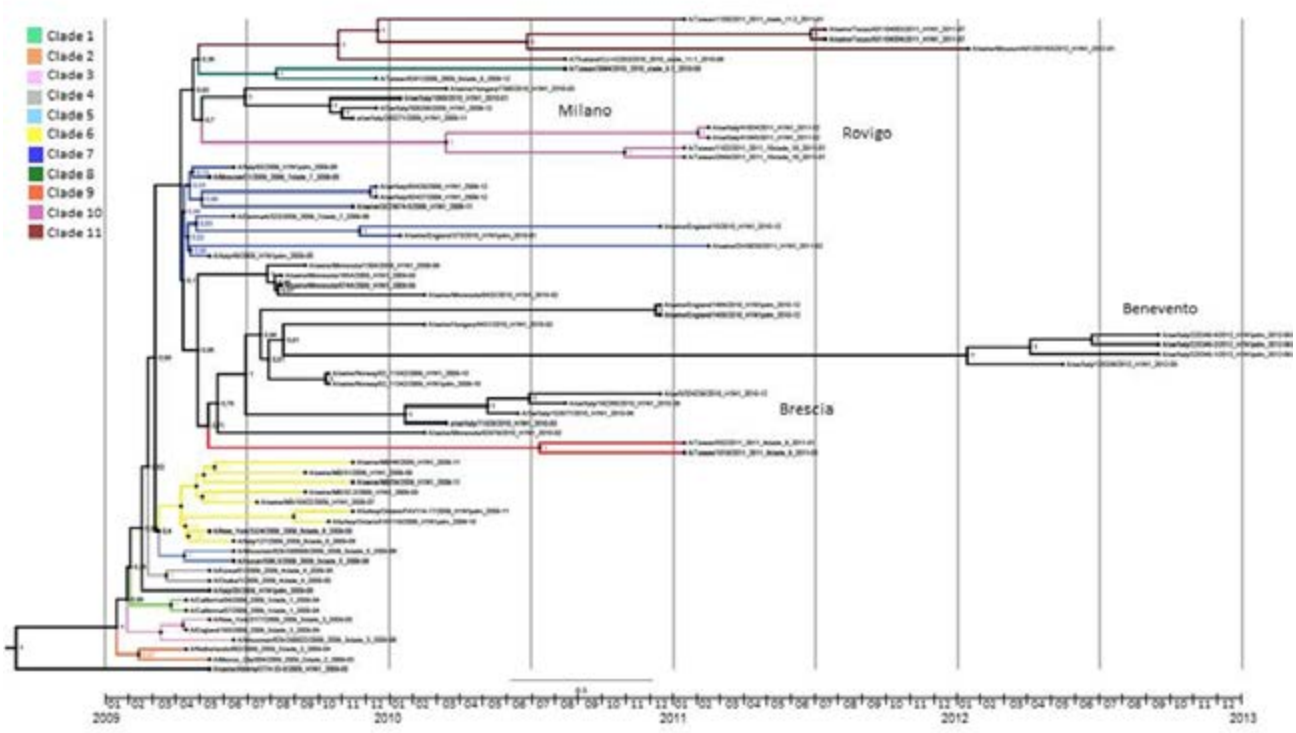
In April 2009, a novel H1N1 (H1N1pdm) influenza A virus was identified as the cause of the present flu pandemic. This virus was generated as a quadruple reassortant, possessing genes from Euro-Asiatic and American lineages of swine influenza, as well as avian and human influenza genes. Swine monitoring programs at IZSLER have been in place since the 1990's and are based on genome detection, virus isolation and sequencing of all respiratory forms. Surveillance performed from 1998 to 2012 revealed a continuous circulation of H1N1, H3N2 and H1N2 viruses and starting in 2009 the isolation of H1N1 pdm viruses in pigs. In 2009-2012, 15 H1N1pdm strains were isolated form 5 different farms located in Milan, Palermo, Brescia, Rovigo and Benevento. This study reports the phylogenetic analysis of the complete genome of the Italian pandemic strains comparing them with 51 H1N1pdm strains from human, swine and turkeys isolated in North America and Europe, divided in 26 human strains, 23 swine from Europe, Canada and US and two turkey strains from Canada.

Only strains for which whole-genome sequences were available and two human strains from the eleven previously described clades (1) are included. The eight segments of 66 whole genomes were manually concatenated prior to phylogenetic tree construction. To infer the evolutionary relationships, we employed a Bayesian Markov chain Monte Carlo method using a strict molecular clock (GTR+I+G4 model),as implemented in BEAST.

The bootstrap supported phylogenetic tree showed that swine and turkey strains clustered in five different clades starting from clade 6, with no isolates placed in the first five clades. Italian isolates were divided in five separate groups depending on the farm of origin. Two isolates from Rovigo and three from Milan belonged to clade 10, but the last ones formed a separate new sub-clade. Four isolates from Brescia and four from Benevento were related to other European swine strains deriving from a common ancestor and were divided in two different new clades. Of these, the most interesting was the one from Benevento characterized by an insertion of two amino acids (aa) in the receptor binding site of HA. The last group of Palermo was the only group not supported by a high bootstrap value; it was closely related to a swine strain originated from Canada and placed in the clade 7

Our analysis showed a clear distinction of Italian strains depending on the farm of origin with no relation between them, probably due to an initial human-to-pig transmission. Subsequently H1N1pdm may undergo many pig-to-pig transmissions because of the continuous availability of susceptible pigs and circulate in the same farm for many months. These results indicated that H1N1pdm virus has distinctively adapted and shared many substitutions. The last detected group from Benevento showed the highest number of aa substitutions including the quite rare double aa insertion in the HA protein

1- Nelson et al., Plos current 2009


Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Clade 6
Clade 7
Clade 8
Clade 9
Clade 10
Clade 11
Milano
Rovigo
Benevento
Brescia
2009
2010
2011
2012
2013

SEROLOGICAL INVESTIGATIONS AGAINST THE MAJOR BOVINE VIRAL RESPIRATORY INFECTIONS

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The aim of this study was to determine the diffusion of the major bovine respiratory viral infections in cattle in Marche Region (Central Italy).

Previous studies have shown the major bovine viral infections in central Italy (1,2). The study involved 21 Marchigiana cattle farms, selected from January to December 2012. 1102 serum samples were collected. The herds were tested in a percentage of animals ranging from 5 to 20%. Sera were used for ELISA tests against Bovine herpesvirus type 1 (BoHV-1); Bovine Respiratory Syncytial Virus (BRSV); Bovine Parainfluenza type 3 (BoPI-3); Bovine Viral Diarrhea Virus (BVDV).

BoHV-1 was detected in fifteen herds. The mean seroprevalence was 44.5% and the positivities,recorded for gE or gB of BoHV-1 respectively, were 33% or 58.8%. Nine herds used different vaccines and had seropositive animals from 43 (animals positive to gE) to 84.5% (animals positive to gB). Ten unvaccinated farms against BoHV-1 had seropositive animals from 8.8 (animals positive to gE) to 13.3 % (animals positive to gB).
BVDV was detected in fourteen herds and the seroprevalence was 46.8%. Four herds were subjected to vaccination and had 39% of seropositive animals, while the remaining eleven herds had a positivity of 52% .
BoPI-3 was detected in seven herds. The seroprevalence was 48.5%. Two herds used different vaccines and had 71% of seropositive animals, while the remaining five herds had a positivity of 42% .
BRSV was detected in six herds and the seroprevalence was 24%. One herd used vaccine and had 67% of seropositive animals, while the remaining five herds had a positivity of 19%.

The results of this study reveal the presence of the major respiratory viruses in Marchigiana cattle herds in the Marche Region. About the prevalence among vaccinated herds, animals showed a positivity percentage ranging from 39 to 84.5%; while among unvaccinated herds the positivity percentage was lower, ranging from 8.8 to 52%. The data observed in this study, are partially in agreement with those obtained by other papers (1,2). In respect to BoHV-1 infection, in this study, a reduction of the seroprevalence in unvaccinated herds has been observed.

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ISOLATION AND CHARACTERIZATION OF Salmonella spp. FROM RED FOXES IN SICILY

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Salmonella species are important zoonotic enteropathogens that affect humans, livestock, companion and zoo animals, and wildlife. In Europe, bacteria of the genus Salmonella cause 31% of cases of food-borne bacterial enteritis in humans. However, despite Salmonella decreases in several countries, Italy reported more human Salmonella cases in 2009 than in 2008, which account for 39.5% of the confirmed cases in Europe. Furthermore, the number of recorded animals with Salmonella is increasing, and many of isolations showed antimicrobial resistance [EFSA, 2011]. The aim of this study was to isolate and characterize Salmonella strains from red foxes hunted or found in Sicily.

120 foxes were collected from January to July 2013 in the Sicilian territory. The isolation of Salmonella spp. was conducted at the Istituto Zooprofilattico Sperimentale della Sicilia according to the ISO standard 6579:2002. The presumptive positive Salmonella isolates were subjected to biochemical analysis using the API 20E identification system. All isolates were serotyped at the National Reference Center for Salmonellosis. Antimicrobial susceptibility testing was carried out on the isolates against 8 antimicrobial agents using the Kirby-Baüer method. We classified the isolates as susceptible, intermediate or resistant according to the Clinical and Laboratory Standards Institute guidelines

33 Salmonella strains wee isolated from Sicilian red foxes. Serotyping of the Salmonella isolates resulted in, 12 S. Manhattan, 7 S. Muenster, 7 S. Montevideo, 1 S. Newport, 1 S. Kambole, 1 S. Tomegbe, 1. S. Richmond, S. Vleuten and 1 S. Derby, 1 S. enterica subsp. Enterica. For all the isolated strains antibiotic susceptibily testing was carried out. All the strains were sensitive to the tested antibiotics (ampicillin, amikacin, enrofloxacin, cefotaxime, tetracicline, co-trimoxazole, chloranfenicol). Only on strain showed an intermediate resistance to tetracicline and ampicillin.

Discussion

Among factors which can have a role in human transmission there are i) carriage by different insects, rodent, and mammals found in close contact with domestic animals; ii) direct contact between human and wildlife; iii) consumption of meat obtained from animals which are carriers of Salmonella (such as waterbirds and wild boards) (Hilbert et al., 2012).

Wildlife may be carriers of several pathogens, which can be transmitted to domestic ruminants, as well as to their farmers. There is a need to increase sampling and testing to ensure a broader evaluation of the epidemiological role of wildlife (Billinis, 2013).

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Discussion

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Billinis C. (2013) Wildlife diseases that pose a risk to small ruminants and their farmers. Small Ruminant Research, 110, 67–70.
EFSA The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009. EFSA Journal 2011;9(7):2154
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TOTAL AFLATOXINS IN CORN: ANALYTICAL PERFORMANCE OF TWO ELISA KITS

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A careful and accurate control of corn for the presence of Aflatoxins (AFs) is crucial since they represent a risk both for agriculture and for human and animal health, in particular AF B1 is recognized as the most potent hepatocarcinogenic of natural origin for human (1). For these reasons accurate, sensitive, reproducible and rapid methods are required to guarantee food safety and the health of the consumer. The aim of this work was to evaluate two commercial ELISA kits (A and B) in terms of reliability and accuracy to establish the level of contamination in corn samples by AFs.

Both kits used had the same analytical procedure (direct and competitive) and also the same range of determination (1-20ppb) and to evaluate the reliability in terms of analytical response, the results obtained from kit analysis, were compared with those obtained by liquid chromatographic analysis (HPLC) of corn samples fortified with AFs in the concentration range of 2.16 to 18.49 ppb (4 points, 4 replicates each). The recovery, the repeatability and the stability of ELISA kits were evaluated, taking into account the performance criteria for methods for determining AFs according to European Regulation 2006/401/EC.

The average of the values of kit B deviated from the statistical point of view, significantly, from those obtained after HPLC analysis, while the curve of the results achieved from analysis with kit A, was comparable to that acquired with chromatography. Kit A showed medium recovery always greater than kit B, characterized by recovery rates next to the minimum performance values required. Kit B therefore underestimates the AFs concentration in corn samples, especially for the lower levels of contamination. Regarding repeatability (RDS%), an index of accuracy, was acceptable at all concentration levels for both kits, respecting the criteria imposed by European Regulation 2006/401/EC (2).
Finally, we evaluated the analytical performance over time of ELISA kits in three different sessions of work, after 10 days apart (T0, T10, T20) and both kits maintained unchanged analytical performance up to 20 days after the opening, if properly stored.

Kit B confirms his lowest reliability and accuracy and a gap to identify positive samples for AFs, in particular at lower levels of contamination. It is therefore essential that companies that provide commercial kits, perform thorough validation tests to ensure the analytical performance required by a screening method to protect the health of the consumer.

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CORTICOSTEROID HORMONE RECEPTORS AND PRERECEPTORS ON HYPOTHALAMUS-PITUITARY-ADRENAL AXIS OF VEAL CALVES.

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Glucocorticoids (GCs) are steroid hormones produced by the adrenal gland and regulated by the hypothalamus-pituitary-adrenal (HPA) axis. GCs signalling in the HPA axis is mediated by the mineralocorticoid receptor (MR) and the glucocorticoids receptor (GR) (1). The action of GCs depends on receptor density, glucocorticoid bioavailability and isozymes activity of the 11beta-hydroxysteroid dehydrogenase (11bHSD). 11bHSD types 1 and 2 are two isoenzymes that convert inactive GCs (e.g., cortisone) to their active forms (e.g., cortisol) and vice versa (2). This study investigate how the prereceptor system and GR and MR react in veal calves experimentally treated with GCs alone or in combination with 17-beta-estradiol (E2), and specifically how is regulated in HPA axis.

The study was carried out on 22 male Friesian veal calves randomly assigned to three experimental groups: Group A (n=8) was administered prednisolone acetate 15 mg/day per os for 31 days; group B (n=6) was administered 5mg of E2 intramuscular, weekly for six times, and 0.4 mg/day of dexamethasone (DEX) per os, for 31 days starting from the second injection of E2. Group C (n=8) was untreated.
Samples of the adrenal gland, hypothalamus and pituitary gland were collected from each animal and immediately placed in nitrogen liquid for further quantitative gene expression. Quantitative PCR of GR, MR, 11bHSD1 and 11bHSD2 was performed.

11bHSD2 is not expressed in hypothalamic tissue of veal calves, whereas is low expressed in pituitary gland. No statistical difference was observed in hypothalamus and pituitary gland for all genes examined in both experimental groups compared to control. In the group A animals the 11b-HSD1 and GR expression were significantly up-regulated in the adrenal gland. Also in group B gene expression was significantly up-regulated in the adrenal gland. In particular, GR and MR gene expression were 4-fold higher than in the controls. In addition, 11b-HSD1 and 2 were up-regulated respectively by 3.5-fold and 1.4-fold higher than in the controls.

The adrenal gland seemed to better respond to E2 and DEX than prednisolone treatment. Both treatments significantly up-regulated 11b-HSD1 and GR gene expression in the adrenal tissues, but only the association of DEX and E2 was able to up-regulate 11b-HSD2 and MR gene. These results partially contrast with the only previously reported study on adrenal glands of male beef cattle treated with GCs (Divari et al., 2011). The reasons for this discrepancy could be attributable to the different age of the animals examined, to a different withdrawal time, and to the co-administration of E2 and DEX. To verify the possible cross talk between the ER and GR in the adrenal gland, in vitro studies of receptors blocking are needed.

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EFFECT OF GROWTH PROMOTERS ADMINISTRATION ON REGUCALCIN GENE EXPRESSION IN THE SEX ACCESSORY GLANDS OF VEAL CALVES

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Regucalcin (RGN) is a Ca2+-binding protein widely expressed in vertebrate. RGN regulates intracellular Ca2+ homeostasis and the activity of several proteins involved in intracellular signalling pathways (1). Recently, RGN was identified as a sex steroid-regulated gene in prostate and testis (2, 3).
This study investigated RGN expression in the sex accessory glands of veal calves experimentally treated with growth promoters (GPs) to establish whether the RGN gene can be considered as a novel biomarker for the detection of GPs abuse in veal calves.

In trial 1 24 Friesian male calves, 6 months old, were divided as follows: gr. A (n=6) treated daily with 1 ml/animal of glyburide (0.2 mg/l) and androsterone (8 mg/l) association; gr. B (n=6) treated weekly with 17β-estradiol for six times until 1 week before slaughter, for a total of 190 mg/animal; gr. C (n=6) treated weekly with testosterone propionate, for a total of 1.050 mg/animal; gr. K1 (n=6) was untreated. In trial 2 30 Friesian veal calves, 6 months old, were divided as follows: gr. D (n=8) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.25 mg/die of brotizolam for 31 days; gr. E (n=8) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.4 mg/die of dexamethasone (DEX) for 31 days; gr. F (n=6) treated with 150 mg/2 weeks of Nandrosol® for 4 weeks and 80 mg/die of ractopamine for 31 days; gr. K2 (n=8) was untreated. Samples of the testis, prostate and bulbo-urethral glands were collected from each animal and preserved for molecular analyses. Quantitative PCR of RGN was performed. Statistical differences were determined by ANOVA, followed by Dunnett's post test, comparing treatment groups against the control group.

High doses of estradiol (gr. B) significantly down-regulated the RGN expression in testis ($P<0.01$), prostate ($P<0.05$) and bulbo-urethral glands ($P<0.01$), whereas testosterone (gr. C) caused a statistical decrease only in testis ($P<0.01$). Conversely, low doses of estradiol significantly up-regulated RGN expression in prostate when associated with brotizolam (gr. D) ($P<0.01$) and DEX (g. E) ($P<0.05$), whereas down-regulated RGN expression in bulbo-urethral glands when associated with brotizolam (gr. D) ($P<0.01$). Only Nandrosol® combined with ractopamine (gr. F) ($P<0.01$) down-regulated RGN expression in testis.

High doses of estradiol down-regulated RGN expression in prostate, as reported in rat (2), whereas low doses had a opposite effect, maybe due also to the association with other molecules like DEX which is able to induce up-regulation of RGN in rat kidney cortex (4).
The specific response of RGN gene expression to the androgen treatment, also at low doses, may suggest its role as a specific androgen-related biomarker in testis to monitoring the prepubertal male veal calves.

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ACETYLSALICYLIC ACID RESIDUES IN BOVINE MILK. DOES AN INDIRECT DETECTION METHOD GIVE A PERSPECTIVE?

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Non-steroidal anti-inflammatory drugs (NSAIDs) are normally used for their efficacy in pain relief and inflammation, to treat mastitis, for long-term analgesia, laminitis and bovine respiratory disease. NSAIDs act as inhibitors of cyclooxygenases enzymes (COX), thus preventing the production of prostaglandins (PGs), prostacyclins (PGI) and thromboxanes (TXBs)(1). These drugs have been regulated in the European Union since 1990 by different laws, recently reorganised within the Commission regulation (EU) n. 37/2010. The aim of the study is to verify the possibility to use an indirect method of screening as a marker of NSAIDs residues in bovine milk. In particular, the acetylsalicylic acid (ASA) administration is not authorised in lactating dairy cows but unofficial sources reveal a massive use to maintain the milk somatic cell count below the legal limits. Therefore, in this study has been tested the real possibility to perform an indirect screening method to detect the presence of ASA residues in bovine milk.

In the present study, the acetylsalicylic acid presence was detected by the measurement of inhibition of TXB2 production. In fact LPS(10microg/ml)-induced TXB2 production, can be inhibited by the presence of ASA's residues in milk (2). The assay was performed by enzyme immunoassay(ELISA)on milk samples from dairy farms of Piedmont Region; all samples was assayed in triplicate.

The acetylsalicylic acid was added at different concentrations (1 microg/ml-10 microg/ml-100 microg/ml)and the obtained result showed an inverse correlation between the presence of ASA in the milk and the TXB2 production. The different TXB2 production was not statistically significant, probably due by a not dose-dependent mechanism.

The results demonstrate that the somatic cells present in the milk sample, are able to generate a response to the LPS-induced inflammatory stimulus; moreover, the presence of ASA in milk is able to inhibit this response. However, more detailed studies should be carried out, particularly by using also other NSAIDs. In fact, all NSAIDs have potential to induce adverse effects, for this reason, the incidental introduction of residues into the human food chain should be avoided and reliable screening method for their rapid determination in animal food products should be developed.

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GLUCOCORTICOID RESIDUES IN BOVINE LIVER

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Dexamethasone (DEX), betamethasone (BETA) and prednisolone (PREDN) are synthetic corticosteroids authorized in cattle breeding for the treatment of metabolic disorders and inflammatory diseases.
In recent years, a widespread use of corticosteroids, as growth promoters, at lower doses than therapeutic ones, has been observed, despite the use of corticosteroids at different indications than those authorized and for purposes other than therapeutic is banned by the EU to safeguard the health of the consumer (Directive 2003/74/EC). During 2007 a surveillance plan was conducted in several slaughterhouses of the Regione del Veneto and about 300 cattle were monitored; specimen of different tissues and organs were collected from 72 animals to detect, through chemical, biomolecular and histological examination, the presence of previous illicit treatments.

Based on the results of the histological analyses on thymus samples, animals were classified into 23 Suspected/Doubts and 16 Negative. Liver samples from these 39 animals were analyzed for the presence of residues of steroids, and urines were analyzed for residues of natural cortisol and cortisone plus synthetic corticosteroids (1). To highlight the presence of corticosteroid residues, a multiresidue method in liquid chromatography coupled with mass spectrometry (LC-MS) has been used to identify and quantify the presence of DEX, BETA and PREDN; the method has been validated for both matrix liver and urine.

The data of confirmatory method adopted, indicated the DEX presence in 11 samples of bovine liver, 10 from the group of 23 animals classified as suspects by histological examination, while only 1 sample out of 16 was not negative; in 6 positive samples, DEX concentrations were higher than the allowed MRL (2 ug/kg). The analytical data of DEX in the livers were in a good correlation with those obtained in urine from the same animals. The 4 positive urine samples were collected from the same animals whose DEX in liver was higher than MRL, and it is likely that, on the basis of literature data on DEX kinetics (2) the treatment with DEX, continued till a few days before slaughter, with no respect of withdrawal times.

The analytical data confirm the high risk for the consumer of foodstuffs of animal origin from cattle illegally treated with glucocorticoids. Based on the results obtained, it can be stated that the histological examination helps to discriminate animals treated with corticosteroids(3).

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DETECTION OF HISTAMINE-PRODUCING PHOTOBACTERIUM DAMSELAE IN FISH

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In 2011, histamine poisoning has been implicated in 78.9% of the foodborne diseases associated with fish consumption in Europe. Food Business Operators have to assess the quality of batches of fish, avoid cross-contamination with potentially hazardous fish species and define the shelf life in order to meet the food safety objectives. Photobacterium damselae subsp. damselae (Pdd) has been detected in fish implicated in histamine food poisonings, but there are not data on histidine decaroboxylase activity (HDC) of strains isolated from fish farmed or captured in Italian seas. With these aims strains of Pdd isolated from Mugilidae captured in the North Tyrrhenian Sea were characterized for their HDC activity and molecular and cultural methods that allow the detection of histamine-producing Pdd in fish were developed.

The eight strains of Pdd used in this study were obtained from IZSTO. Microbial suspensions were incubated at 30°C for 24 h in modified tryptone soy broth containing histidine (TSB+) and electrochemical measurements of histamine were carried out by an enzyme biosensor, which was constructed by immobilizing diamino-oxydase and horseradish peroxidase on the surface of a graphite electrode. Two Pdd strains were inoculated on fresh mackerel fillets purchased from a local supermarket. These were divided in three portions. One was used as control and the other two were inoculated with different level (4 and 40 cfu/g) of Pdd. The amount of histamine was measured in samples enriched in TSB+ at 25°C for 48 h. Aliquots of the enriched cultures were inoculated in thiosulfate-citrate-bile salts-sucrose agar (TCBS) and colonies with typical morphology were transplanted on Niven agar. DNA extracts of bacteria fermenting galactose and glucose were assessed by PCR to detect the species specific genes ureC and 16S rRNA. A molecular method that allows detection of histamine producers Photobacterium damselae subsp. damselae by PCR was developed.

All strains were strong histamine producers, yielding concentration varying from 955 and 8,977 mg/kg in TSB+ cultures. Enriched cultures of the fish samples inoculated with Pdd had high histamine concentrations (8500–8900 ng/ml). Isolated colonies displaying typical morphology on TCBS agar and Niven agar were constantly detected from the inoculated fish samples. PCR assay for ureC and 16S rRNA discriminate Pdd from other isolates showing similar morphology. The primers directed toward the conserved region of HDC gene of Gram- bacteria failed to produce specific PCR products. The HDC gene was successfully amplified from DNA of all Pdd strains by specific primer pairs corresponding to the base pair position 54 to 73 and 375 to 394 of the HDC gene of Pdd.

Strains of Pdd isolated from Mugilidae captured in the North Tyrrhenian Sea were strong histamine producer. The analytical procedure developed can detect Pdd in fish and use of histamine biosensor help to detect contaminated batches.

No bibliography

NORTHERN ITALY DISTRIBUTION OF TICK INFESTING WILD FAUNA AND POTENTIAL VECTOR OF TICK-BORNE DISEASES.

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Emilia Romagna and Lombardia regions are located in the northern part of Italy. Various tick-borne diseases (TBD) occur in this area, where Lyme disease, transmitted by Ixodes ricinus, is frequently diagnosed in humans (1). This work is an attempt to gather suitable data for surveillance on Ixoxid fauna of wildlife hunter-killed animals and for risk assessment on tick-borne diseases in our regions.

Diagnosis of TBDs increased over the last years and attention of public health services focused on emerging vector borne diseases and wild fauna transmitted diseases. In this study, Ixodid ticks were collected from wild animals after being hunter-killed or found dead and submitted to IZSLER laboratories for necropsy during 2008-2012, from two Italian region, Lombardia and Emilia-Romagna. Ticks were removed and identified following Manilla (2) taxonomic keys and mapped using an open source software (QuantumGis) at municipality level.

A total of 6675 tick exemplars were collected from 268 municipalities in five years of surveillance. We sampled a total of 13 different mammals species: the most sampled animals were hunted game animals like roe deer (Capreolus capreolus), wild boar (Sus scrofa) and hare (Lepus europaeus). Mean tick intensity (mean number of ticks per host) was determined in each host species: the greater number of ticks per host was on hedgehog (t.i.=15,7) and hare (t.i.=10,6). Tick infestation differ between host species ($p<0.05$, chi2): wild boar was found infested by eight tick species, followed by fox (infested by seven species). Dermacentor marginatus is significantly associated ($p<0.05$, chi2) to wild boar and is rarely observed on other mammals, while I. ricinus was collected on all mammal species considered but with different abundance; in roe deer and hare about 90% of ticks collected were I. ricinus.
We found tick species that are vectors of many TBD: I. ricinus represent the main tick species (N=3939; 59%) and it's well distributed in all northern Italy. Other vectors are Rhipicephalus sanguineus (n=891; 13%), I. hexagonus (n=669; 10%), R. turanicus (n=596; 9%), D. marginatus (n=439; 7%) and Hyalomma marginatum (n=37; 0,6%).

Historical data on presence/abundance of disease vectors are essential to understand TBD epidemiology and to gather data for diseases transmission. Our surveillance system allows to map geographic distribution of ticks species at municipality level and provide a good base to detect and respond to vector-borne diseases related health threats.

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MULTIRESISTANT SALMONELLA TYPHIMURIUM MONOPHASIC VARIANT 1,4,[5],12:I:- ISOLATED FROM SLAUGHTER PIGS

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Aims of the present study were to provide preliminary data on prevalence of Salmonella serovars isolated from pigs at the slaughterhouse and to evaluate the patterns of resistance of Salmonella enterica serovar 1,4,[5],12: i:-, a monophasic variant of Salmonella Typhimurium.

From February to June 2013, a cross-sectional study was performed on 16 batches of heavy pigs (9-10 months old, 160 kg BW) randomly sampled at slaughterhouse. To calculate the prevalence of Salmonella spp., for each batch (n=135 pigs on average) 30 samples of ileocecal lymph nodes (ILN) (n=480) were collected and analysed by ISO 6579:2002/Amd 1 Annex D:2007. Considering the intra-batch prevalence, a representative number of Salmonella enterica serovar 1,4,[5],12: i:- isolates, for each batch, were tested for the antimicrobial susceptibility to several antibiotics: ampicillin (A), chloramphenicol (C), streptomycin (S), sulfamethoxazole (Su), tetracycline (T), florfenicol (FFN), kanamycin (KAN). The antimicrobial susceptibility was tested using a broth microdiluition procedure in accordance with the CLSI guidelines. Intermediate isolates were grouped with susceptible isolates.

Salmonella spp. was detected in 86 out 480 ILN (17.9% CI95% 14.4%-21.4%). A total of 11 different serotypes of Salmonella were identified (Table 1). S.1,4,[5],12:i:- was by far the most prevalent serotype, identified in 48.8% of the positive pigs, followed by S.Goldcoast (20.9%), S.Livingstone and S.London (8.1%). Fifteen batches out of 16 were positive and intra-batch prevalence ranged from 3.3% to 66.7% (CI95% 0.1%-81.8%).
Multiresistence, defined as the resistance to four or more antimicrobials, was identified in all the 23 strains tested. The resistance pattern ASSuT was showed in 15 isolates and penta-resistance profile ACSSuT was found in 8 isolates. The ASSuT strains presented additional resistance to KAN in 6 cases, while all ACSSuT strains were also resistant to FFN.

The prevalence of Salmonella serovars reported in this study showed an overall decline of serovar Typhimurium. To some extent this reduction has been counteracted by an increase in prevalence of serovar 1,4,[5],12:i:- isolates with ASSuT and ACSSuT profile [1]. In contrast to the monophasic variants isolated in Thailand and Spain, which commonly expressed additional resistance to gentamicin and trimethoprim-sulphametoxazole and/or chloramphenicol [1], in our study an additional resistance of tetra-resistant and penta-resistant Salmonella enterica serovar 1,4,[5],12: i:- was observed to KAN and FFN respectively.

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Table 1. Serovars of S. Enterica detected in slaughter pigs			
S. Enterica serovars	**Positive ILN (*n*)**	**% Isolates**	**Number of positive batches**
1,4,[5],12:i:-	42	48,84	9
Goldcoast	18	20,93	1
Livingstone	7	8,14	3
London	7	8,14	2
Typhimurium	2	2,32	2
Derby	2	2,32	1
Branderburg	2	2,32	1
Kapemba	2	2,32	1
Bradney	2	2,32	1
Mishmarhaemek	1	1,16	1
Thompson	1	1,16	1
Total	86		16
ILN-ileocecal lymph nodes			

ANTIMICROBIAL RESISTANCE IN SALMONELLA TYPHIMURIUM AND MONOPHASIC VARIANT SALMONELLA ENTERICA SEROVAR 1,4,[5],12:I:- FROM WILD BIRDS IN NORTHERN ITALY

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THE AIM OF THE WORK was to evaluate the patterns of resistance of Salmonella Typhimurium and monophasic variant Salmonella enterica serovar 1,4,[5],12:i:- strains isolated from wild birds in Northern Italy during the period 2006-2012.

Sampling: 35 strains of Salmonella Typhimurium and 5 strains of monophasic variant Salmonella enterica serovar 1,4,[5],12:i:- isolated during the period 2006-2012 from wild birds were included in this study. Antimicrobial susceptibility testing: the strains of Salmonella mentioned before were tested for their susceptibility to a panel of 14 antimicrobials (ampicillin-AMP, apramycin-APR, ceftiofur-EFT, chloramphenicol-C, enrofloxacin-ENR, florfenicol-FFC, gentamicin-CN, kanamycin-K, nalidixic acid-NA, neomycin-N, spectinomycin-SH, streptomycin-S, sulphonamide-Su and tetracycline-TE) using the disc diffusion method following the procedures of the Clinical and Laboratory Standards Institute (2002, 2006).

Eighteen Salmonella Typhimurium strains resulted sensitive to all antibiotics used in this study, 9 strains showed resistance to one antibiotic only while 8 strains showed multidrug resistance (resistance to four or more antibiotics). The prevalent pattern of resistance was ASSuT (ampicillin, streptomycin, sulfonamide and tetracycline), observed in 23% of the strains. Four strains of the monophasic variant showed multidrug resistance and the patterns observed were ASSuT (2 strains) and ACSSuT (2 strains).

Salmonella Typhimurium and monophasic variant are commonly isolated from mammals, birds and human cases of gastroenteritis. In general the majority of Salmonella isolates exhibiting multidrug resistance. Our study showed that 23% of the Salmonella Typhimurium strains and 80% of monophasic variant strains were resistant to four or plus antibiotics and that these two serotypes exhibit one main multidrug resistance profile: ASSuT. This pattern of resistance has recently emerged in Italy in strains of Salmonella Typhimurium and in monophasic variant strains. Strains showing the ASSuT profile have also been commonly isolated from animal sources, in particular swine. While the frequency of the ACSSuT pattern among the Italian human isolates of Salmonella Typhimurium remained constant over the years, the frequency of the tetra-resistant pattern increased from 7% in the period 1999-2001 to 34,1% in 2006, when the penta-resistant strains accounted for 29,6% (1). This study confirms the usefulness of antimicrobial resistance to follow the emergence and spread of multidrug resistant strains of Salmonella.

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THE USE OF FORENSIC ENTOMOLOGY IN VETERINARY MEDICINE: SCIENTIFIC AND TECHNICAL SUPPORT IN JUDICIAL INVESTIGATIONS

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The forensic entomology can be used for the calculation of the Post-Mortem Interval (PMI) in medical legal investigation both in the human and veterinary field. In recent decades numerous are the cases of judicial finding of animal carcasses on which are needed to determine the time of death. This abstract describes one case.

On March 3 2010 3 carrions of sheeps, 3 carrions of pot-bellied pig, 3 carrions of Dromaius novaehollandiae and 1 carrion of Canis lupus familiaris at different decomposition stages were collected by veterinarian of Provincial Veterinary Service of Ferrara, in farm located in San Bartolomeo in Bosco (FE) together with Several insects (both larval and adults stage) and sent immediately to Istituto Zooprofilattico Sperimentale of Lombardia and Emilia Romagna (IZSLER), , for diagnostic investigations and to determine the time of dead. The insects sampled were: beetles (Staphilinidae, Dermestidae and Tenebrionidae) on the carcasses with the most advanced decomposition stages i.e. on every pot-bellied carrions, on one Dromaius novaehollandiae, and on one sheep; instead only Diptera (Calliphoridae, Stratiomidae and Piophilidae) were find on other carrions. The larvae were reared to adulthood. Time of hatch, together with species identification, macro and micro climate and lab developmental data were used to determine the time of death. We used two methods of dating the colonization: the Isomorphen-Isomegalen diagrams and the ADD concept [1 and 2].

The entomological evidence has allowed to date the death of different animal carcasses and to establish that the first animal has died on October 2009 while the last one died during the second half of February. The time was consistent with the time that the defendants were seen at the scene and was used to define the time spent by the abandonment of animals, without food. This case highlights such as entomological survey become powerful evidence for the length of time of abuse or neglect, in court.

Forensic practice applied to pets is increasing in importance and in the veterinary profession has a key role to play. There is a need to enhance the awareness of veterinarians, to introduce teaching and specialized training and provide access to information accordingly. Systems and protocols, some similar to those used in human forensic medicine, must be established and used in veterinary cases. In this scenario the veterinary forensic entomology is not yet a recognized discipline but it is evolving rapidly.

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FLOTAC FOR URO-MICROSCOPIC DIAGNOSIS OF CAPILLARIA PLICA IN DOGS

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Capillaria plica (Syn. Pearsonema plica), commonly known as the “bladderworm” is a nematode that resides in the urinary bladder and rarely in ureters or in the kidney pelvis of various wild carnivores, especially foxes and dogs (Bork-Mimm and Rinder, 2011). Urinary sedimentation technique is actually the only diagnostic tool that permits the identification of C. plica eggs. The aim of this study was to compare two innovative techniques, FLOTAC and Mini-FLOTAC, with the “classical” technique of sedimentation for the diagnosis of C. plica in dog urine.

A 4-year-old Labrador Retriever, male, from the Apulia Region (southern Italy) was presented to the referring clinician with macrohaematuria. A first examination of urinary sediment revealed the presence of several C. plica eggs.
Two aliquots of 200 ml of urine were collected from the dog infected by C. plica. Each aliquot of urine was accurately homogenized and divided in 18 tubes each filled with 10 ml of urine, to have 6 replicates for each diagnostic method.
The tubes were randomly assigned to the following techniques: FLOTAC (Cringoli et al., 2010), Mini-FLOTAC (Cringoli et al., 2013) and sedimentation (WHO, 1991). A sodium chloride-based flotation solution (FS2, specific gravity= 1.20) was used for the FLOTAC and Mini-FLOTAC techniques. The analytic sensitivity of each technique was 1 egg per 10 ml of urine.

All the three techniques were capable to detect C. plica eggs. For aliquot 1, the mean number of C. plica detected with FLOTAC was significantly higher than those detected by Mini-FLOTAC and sedimentation (40.7 eggs per 10 ml of urine vs 28.3 and 20.7, respectively); however, the CV% detected with FLOTAC was lower (5.5 vs 12.8 and 36.0, respectively). Also for the aliquot 2, FLOTAC gave higher mean than the other two techniques (99.8 eggs per 10 ml of urine vs 52.3 and 44.8, respectively) and lower CV% (6.8 vs 14.0 and 29.7, respectively).

The findings of the present study suggested that FLOTAC is the best method for the diagnosis of C. plica in dog urine. An alternative diagnostic method is Mini-FLOTAC that can be used in place of FLOTAC in laboratories where the centrifugation step cannot be performed.

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PARASITOLOGICAL AND CLINICAL FINDINGS OF TRITRICHOMONAS FOETUS IN A CAT.

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Tritrichomonas foetus is a small flagellated protozoa well-known as venereal pathogen of cattle. In the last years it has been identified worldwide as a causative agent of large bowel diarrhea in cats, even if it is not commonly diagnosed. Clinical signs can be variable, from subclinical infection to chronic intermittent diarrhea. We are reporting a case of clinical trichomoniasis in a cat which has been presented to the Department of Veterinary Medical Sciences of Bologna University (Italy).

A 6 months years old Main Coon male cat was presented for a chronic intermittent diarrhea with semi solid stools;the symptoms appeared and persisted since it was taken home from the cattery. The animal was previously treated with different drugs (neomycin, bacitracin, febendazole and diosmectite) without any clinical improvement and tested Giardia sp. negative. Physical exam, abdominal ultrasound, hematologic and chemistry profile were performed. A fecal sample was collected using a rectal swab and analyzed by a direct saline smear under 100X magnification; a thin Giemsa and May-Grumwald Giemsa- stained fecal smear was prepared for the morphological analysis of the organisms eventually present. The fecal sample was tested by the ITS nested PCR described by Goodkin et al., (2002), specific for the detection of T. foetus in feline fecal samples.

The physical exam did not reveal any significant abnormality; the abdominal ultrasound and the blood work showed an inflammatory pattern of the large bowel associated to regional lymphadenopathy and a mild eosinophilia (1610/mm3; range 0-750/mm3), respectively. The direct saline smear revealed the presence of motile organisms that were identified as belonging to Trichomonadida based on morphological characters observed in stained smear. The PCR resulted positive.

T. foeuts was identified as the causative agent of the large bowl diarrhea in the cat. The cat was put on metronidazole (15 mg/kg BID per os) with an immediate clinical improvement: the consistency of the feces returned to normal. The suspension of the treatment caused a relapsed of the clinical signs and metronidazole was restarted. The cat improved again and it was possible to gradually discontinue the drug. However, due to the poor compliance of the owners, a long term follow up is missing. Trichomoniasis is a parasitic disease that is still not commonly diagnosed; the diagnosis is precluded by routine flotation techniques, delayed examination and refrigeration of the feces as T. foetus organisms are fragile. Thus it is important to consider this parasite as a possible etiologic agent in cats with a history of chronic large-bowel diarrhea; further investigation on its diffusion in the feline population in Italy should be needed.

Goodkin et al.(2002). Single-Tube Nested PCR for Detection of Tritrichomonas foetus in Feline Feces. JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 4126–413.

HISTOPATHOLOGY AS DIAGNOSTIC TOOL FOR HONEY BEE DISEASES.

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A broad spectrum of specific pathogens affects the honey bee colony including bacteria, viruses, fungi and parasites.Knowledge of the biology and epidemiology of these pathogens is needed for prevention of disease outbreak.During recent years,the use of molecular methods has increased offering a selection of powerful tools for diagnostics laboratories involved in honey bee diseases.To the best of our knowledge, histopathological method has never been used so far for the diagnosis of disease in honey bee colonies, even if it remains often the most important diagnostic method for human and animal diseases.

Larvae (50) and adult honey bees (50) were collected from colonies without signs of disease in the Campania region.One half of samples was used to examine the presence of Nosema spores, the other half was 10% formalin fixed, paraffin wax embedded and stained with haematoxylin-eosin and with histochemical methods (PAS and Grocott).Before processing samples were observed under the stereomicroscope and then were necropsied.

The stereomicroscopic observation did not reveal any pathological changes,differently to the histological examination.Some young drone larvae presented numerous hyphal filaments that invaded,destroyed and replaced all the larval tissues.The hyphae, identified as Ascosphaera apis by Grocott's technique, were septated, 2.5-8 µm in diameter, showed pronounced dichotomous branching and released numerous conidia(Maiolino,2013).Some adult honey bees revealed the presence of different parasitic stages such as mature and immature spores all along the ventriculus epithelium and in the gut lumen. Light microscopy revealed that Nosema spores were regular, oval shaped, highly refractive and with a dark halo around them (Higes,2007; Chen,2009). These characteristics were not sufficient to tell apart the spores of Nosema spp.

Considering the increase in incidences of many diseases of the honey bees in many parts of the world, including Italy, to have a sensitive and rapid technique for the detection of the causative agent is important.Our results demonstrated that the histopathology can be a sensitive and efficient method for the detection and identification of numerous honey bee pathogens and can have the additional advantage of provide a diagnosis in colonies without signs of disease.

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ESCHERICHIA COLI FROM HEALTHY ANIMALS AND FOOD-PRODUCTS OF ANIMAL ORIGIN AS RESERVOIR OF ANTIBIOTIC RESISTANCE AND VIRULENCE

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Escherichia coli is a commensal bacteria in the gastrointestinal tract of humans, but some strains cause diseases ranging from diarrhea to extra-intestinal infections under certain condition. Diarrheagenic E. coli strains are acquired by consuming contaminated food or water, therefore, food contamination is a major public health concern. Antibiotic resistance in E. coli from human or animal origins has been increasingly reported worldwide, and it is a cause of concern. The aim of this work was to study a collection of E. coli from animal origins in order to investigate antibiotic susceptibilities, genes encoding antibiotic resistance and virulence factors as well as their phylogenetic groups.

39 isolates of E .coli were collected from healthy animals and food-products of animal origin in Tunisia. The isolated were incubated at 37°C for 18 to 24 hours on MacConkey agar. Antibiotic susceptibility was carried out by disc diffusion method according to CLSI guidelines. Phylogenetic groups of E. coli (A, B1, B2, and D), genes encoding antibiotic resistance, and a set of 32 genes encoding virulence factors were investigated using PCR technique following the protocol described in literature.

High rates of antibiotic resistance were detected for ampicillin, tetracycline, trimethoprim/sulfamethoxazole, gentamicin, and streptomycin as well as multidrug-resistant isolates. The tetA and tetB genes, encoding tetracycline resistance were detected in 8 and 7 isolates, respectively. However, phenotypic resistance was not detected in 3 tet-harboring isolates. Sulfonamide resistance was encoded by sul1, sul2, and sul3 genes in 1, 4, and 1 isolate, respectively. Interestingly, plasmid mediated quinolone resistance was detected in 6 isolates, where qnrS was in 3, qnrB in 2 and qnrA in only one strain. The majority of isolates belongs to the phylogenetic groups A and B1, considered as commensal isolates. Genes encoding virulence factors fimH (type 1 fimbria), cvaC (colicin V), traT (serum resistance), fyuA (yersiniabactin), and east1 (enteroaggregative heat-stable enterotoxin) were harbored by 36 (92.3%), 2 (5%), 10 (25.6%), 10 (25.6%), 16 (41%), respectively. Interestingly, the ipaH (invasion plasmid antigen), specific for enteroinvasive E. coli (EIEC), and ibeA (brain microvascular endothelial cells invasion), were detected, each, in one isolate.

These finding showed the importance of food-products of animal origin as reservoir of antibiotic-resistant and virulent E. coli isolates.

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5-LIPOXYGENASE (5-LOX) IN EQUINE CYATHOSTOMINOSIS: BIOCHEMICAL INVESTIGATIONS

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The aim of this work was to evaluate the role of the enzyme 5-lipoxygenase (5-LOX), in the pathogenesis of morpho-functional damage in equine cyathostominosis, a major parasitic disease of horses. Previously, this enzyme has been studied in the porcine parasitic bronco-pneumonias (1).

Intestinal samples were collected from horses bred in Italy and France for human consumption, and with cyathostomin infection. The resistance status with regard to benzimidazolics was evaluated via the Faecal Egg Count Reduction Test (FECRT) and the horses were subsequently subjected to three different therapeutic regimens according to each group as on the follows: single dose (10 mg/kg PV per os) of Fenbenzadole (FBZ), repeated dosage (10 mg/kg PV per os) of FBZ per 5 days (FBZx5) and single dose (0.4 mg/kg PV per os) of moxidectin (MOX). Subsequently and based on the response to the anthelmintic treatment, the horses were divided into two groups: non-infected (n=8) and infected (n=8) with encysted cyathostomin larvae in the intestines detected at the slaughterhouse. 5 non-infected horses had previously been treated with FBZ5X (n.1), FBZ (n.2) and MOX (n.2). Of the infected animals, 4 had been treated with MOX, 1 with FBZ and 1 with FBZ5X. Tissue samples from the cecum, dorsal and ventral colon were taken from each animal and immediately refrigerated and subsequently frozen and stored at -80°C until analysis. The expression of 5-LOX was assessed by immunoblotting,and the enzymatic activity was measured with a functional test. Statistical analysis was performed by T test and 1-way ANOVA (GraphPad Prism 5, San Diego, CA, USA).

No significant differences between the cecum samples from non-infected and infected animals were detected in the expression and the activity of 5-LOX. In the ventral colon of infected animals a significant increase in the enzyme expression was noticed, and an increasing trend only in the enzymatic activity. In the dorsal colon of the infected animals a significantly higher level of enzymatic expression and activity was found. A significant increase in the enzyme expression and activity was shown in the cecum and dorsal colon compared to the ventral colon in the infected animals, while non-infected animals showed a significantly higher level of 5-LOX expression and activity in the cecum compared to the other two areas of the colon. There are no differences between the horses groups treated with different protocols.

These results indicate that 5-LOX is expressed at highest levels in those horses harbouring encysted cyathostomin larvae in the large intestine which derive from FBZ-resistant populations, rather than susceptible isolates, thus suggesting an involvement of this enzymatic pathway in the pathogenesis of equine cyathostominosis.

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GENOMIC CHARACTERIZATION OF H3N2 SWINE INFLUENZA A VIRUS ISOLATED IN CENTRAL ITALY

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The aim of this study was to characterize genetically a swine influenza virus (SIV) isolated in 2012 from a swine herd of Umbria region.

Nasal swabs were collected from pigs showing clinical signs and/or lesions related to swine influenza. Samples were inoculated on Newborn Swine Kidney (NSK) cells and through the allantoic sac route of 9- to 11-day-old SPF chicken embryonated eggs. The cell culture supernatant and allantoic fluid were tested by hemagglutination assay using chicken erythrocytes using the standard procedure. Viral RNA was extracted using the QIAamp ViralRNA Mini kit and tested for influenza A virus (1) and H1N1 pandemic by different real-time RT-PCR and then subtyped by two multiplex RT-PCR assays(2). Partial sequence of matrix (M) gene and the full length of the hemagglutinin (HA), neuraminidase (NA) and nucleoprotein (NP) genes were performed for genomic characterization (3, 4). Both the sense and antisense trends were sequenced performing three independent clones for each sample. Sequence data were analysed using the program SeqMan II v 5.07 from the DNASTAR package and editing with BioEdit Sequence Alignment Editor v 7.0.9.0 in respect of the amino acidic coding frame and were aligned with homologous sequences available on GenBank. A maximum-likelihood tree was inferred for each of the datasets using the PhyML program v 3.0, statistical support for individual nodes was estimated by bootstrap analysis (10,000 replicates). The phylogenetic tree was analyzed by FigTree v 1.3.1.

Nasal swabs were positive by both the virus isolation techniques and by real-time RT-PCR for influenza A virus. Negative results were obtained by real-time RT-PCR H1N1 pandemic. Two multiplex RT-PCR, specific for HA and NA genes, were found to be positive for influenza A virus H3N2 subtype. The phylogenetic trees constructed with the four datasets showed that the isolate belongs to the swine European H3N2 cluster. In particular, the phylogenetic tree based on NP and M genes, confirmed that the virus isolate analyzed was an European Avian-like SIV (5). Finally the phylogenetic tree constructed with the HA dataset showed that the isolate was closely located to the European Human H3N2 cluster.

Further investigations will be necessary to verify the genotype patterns of this isolate and to established the correlations with contemporary reassorted H3N2 viruses described recently in US swine population (6).

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CASE OF LISTERIA MONOCYTOGENES CONTAMINATION IN RAW MILK FOR HUMAN CONSUMPTION: MULTIDISCIPLINARY INVESTIGATION

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The study describes an investigation conducted in a dairy cattle farm,through multidisciplinary approaches, in order to identify the origin of contamination of bulk raw milk by L. monocytogenes.

During an official control program on raw milk, directly distributed for human consumption, bulk tank milk of a farm was found positive for L. monocytogenes by Real-Time PCR (AFNOR validated - Biorad) and subsequent isolation (EN ISO 11290-1:1996). Single bovine milk and environmental swabs were collected and analyzed in order to investigate the source of contamination. Pulsed-Field Gel Electrophoresis (CDC PulseNet protocol) and Ribotyping (DuPont) were performed for comparing all the isolates. Virulence genes were detected by PCR as previously described 1,2 .

After detection of L. monocytogenes in bulk tank milk of the farm, a deep sanitization of the robotic milking system was performed, without any effect on milk contamination. Analysis of single bovine milk samples, led to the identification of an animal shedding L. monocytogenes from its rear left quarter. The infected cow showed no clinical signs of disease. Nevertheless, since the beginning of lactation, slightly high somatic cell counts had been reported. Intravenous and intramammary antibiotic treatment was ineffective, so the bovine was culled. No abnormalities of internal organs were observed at post mortem examination. Histological analysis revealed signs of interstitial mastitis in three mammary quarters. L. monocytogenes was not detected by immunohistochemistry in any specimens, while was isolated, from mammary parenchyma, mammary lymph nodes, kidneys, spleen, urine, ruminal and intestinal content. After removal of the infected cow, some daily bulk milk samples and in-line milk filters were still found positive for L. monocytogenes. Milk of every cow of the herd was re-tested and confirmed negative. Swabs from the milk equipment and farm environment led to detection of L. monocytogenes in the faucet used to empty the tank. Only after a deep sanitization of the faucet, bulk milk became definitively negative. PFGE and Ribotyping revealed a high relation between the isolates, which were also demonstrated positive for genes coding for the main virulence factors by PCR.

The high similarity among L. monocytogenes isolates, demonstrated by PFGE and Ribotyping, supports their clonal origin, suggesting that the infected cow was the cause of colonization of the tank faucet, which in turn was responsible of persistence of L. monocytogenes in bulk milk after the removal of the animal.

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DETECTION OF HEPATITIS E VIRUS IN SWINE LIVER SAUSAGE, IN ITALY

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Hepatitis E is an infectious disease caused by a small RNA virus (Hepatitis E Virus, HEV), which can cause acute hepatitis in humans. Four mammalian HEV genotypes are known, among which g3 and g4 are considered zoonotic.
In developed countries, hepatitis E cases were mostly associated with travelling in endemic areas. However, recently autochthonous cases have been increasingly reported in developed country, including Italy.
In Italy, g3 strains have been detected in swine, in both farms and slaughterhouses, with prevalence that can reach 50% (2,3). Swine strains often share a high nucleotide identity with HuHEV.
During the last 10 years, hepatitis E cases have been correlated with consumption of swine, wild boar and deer meat or organs (particularly liver) consumed raw or undercooked, worldwide (1).

In this study, we investigated the presence of HEV in liver sausage, which is often consumed raw or undercooked in Italy. In 2012, 4 packages (300 gr) of pork liver sausages were bought at a butcher shop. Sausages were chopped in 45 slices (250 mg each) and spiked with Murine Norovirus (MNV). After RNA extraction, samples were analyzed by RT-PCR for MNV detection (extraction process control). Samples positive for MNV were then analyzed for HEV and Porcine Adenovirus (PAdV as index virus of fecal contamination) by real-time RT-PCR and real-time PCR, respectively. Positive samples were further analyzed by RT-PCR with specific primers for two different genomic regions (ORF1 and ORF2).

Two out of 45 samples (4.4%) were positive for HEV. Moreover both samples were positive for HEV ORF1, and one was also confirmed in ORF2. This result suggests different sensitivity of the two PCR protocols.
Sequence analysis confirmed the presence of swine g3 HEV strains in both samples. One sausage sample was positive for HEV and PAdV, suggesting that also HEV presence could be related to fecal contamination of pork liver. Attempts to infect A549 cells with a homogenate from a HEV and PAdV positive liver sausage were unsuccessful.

This study confirms that pig liver sausage can be contaminated with HEV, which might at least partially reflect fecal contamination of pork meat and liver during improper slaughtering practice. Further studies to investigate residual HEV infectivity in pork are needed.

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RT-PCR AND IHS EVALUATION OF CANINE DISTEMPER VIRUS (CDV) AND PHOCINE DISTEMPER VIRUS (PDV) LESIONS IN CANINE AND MARINE MAMMALS TISSUE SAMPLES

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Morbillivirus, family Paramyxoviridae and order Mononegavirales, are RNA viruses with a nucleocapsid core containing a single-stranded, non-segmented RNA genome of negative sense of 15 to 16 kilobases. This virus can cause well known diseases, such as measles and rinderpest, highly infectious. Until 1988, many other species of morbillivirus which cause infections in terrestrial mammals, including humans, were identified. In recent years, there have been several epidemics affecting phocine and marine mammals such as whales, which led to the identification of several new viral species. These are highly contagious viral diseases requiring different diagnostic approaches to better evaluate its pathogenesis. To highlight the presence of Canine Distemper Virus (CDV) and the Dolphin morbillivirus (DMV) in tissue samples (previously subjected DMV antigen detection using a rabbit hyperimmune polyclonal serum against DMV), one step RT-PCR and heminested in liquid phase PCR were performed.

PCR protocol was applied using specific primers for a portion region of nucleocapsid encoding gene protein of CDV, designed by Frisk et al. (1999). Among these, primer forward N1a has been degenerated to amplify a conserved region of the N gene of both CDV and DMV. RNA was extracted from the tissues according to different procedures depending on the type of fixation whether the tissue was fresh, frozen or formalin fixed. A 137 bps cDNA probe for in situ hybridization was then used to ascertain the presence of the virus in two, a canine and a marine mammal, RT-PCR positive subjects.

Nine samples collected from different organs of 9 dolphins were positive (for a total of 4 positive subjects), as for 17 samples collected from different organs of 9 dogs (8 positive subjects). In situ hybridization of two samples tested showed a clear positivity.

The results clearly showed the presence of the virus, in particular in the brain and lung, and confirmed IHC findings. The weak positivity detected in the tissues suggests that the use of more specific and sensitive methods is a valuable diagnostic support to characterise different Morbillivirus strains.

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SALMONELLA TYPHIMURIUM EXPLOITS INFLAMMATION TO MULTIPLY AND SURVIVE IN PIG

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Salmonella spp. is one of the most common food-borne zoonosis in the European Union and, although poultry is recognized as the major source, the contribution of pigs to human infection is relevant. Recently, a percentage of 26.9% (95% CI 26.3-27.6%) of human cases was epidemiologically attributed to consumption of pork products in Europe, being a 10.3 % of slaughtered animals carrying bacteria in lymph nodes. This scenario is cause of concern among food safety Authorities and prompts responses aiming at reducing the most prominent serotypes of Salmonella entering the human food chain.
Infection of pigs with the swine adapted S.Typhisuis and S.Choleraesuis usually result in swine typhoid, characterized by an often fatal severe systemic disease. Conversely, the broad-host range serotypes of Salmonella spp., and in particular S.Typhimurium, usually are asymptomatic, but may result in enteric disease and generally are followed by a long-lasting colonization in tonsils, gut and gut-associated lymphoid tissue. Although, depth information is available about the pathogenic mechanisms of host-adapted Salmonella spp., little is still known about the broad-host range serotypes in pigs.

On that account, we performed a series of experiments comparing the host-pathogen interaction in post-weaned piglets of 25 days of age, orally inoculated with fully virulent or attenuated S.Typhimurium.

Using this model of infection, we found that oral infection induces a rapid colonization of different organs that determines a clinical condition closely related to the virulence of the bacteria. In particular, we found that both virulent or attenuated S.Typhimurium colonize tonsils and gut-draining lymph nodes as early 24 hours after oral inoculation. In addition, we provided evidence that virulent S.Typhimurium induces a local and systemic inflammation, characterized by fever and proinflammatory cytokines, which in a separate set of experiments was ascertained to favor pathogen survival in lymphatic organs.

This information shows that pig is a suitable model to study salmonellosis and provides new insights about the pathogenic mechanisms of salmonellosis.

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RESPIRATORY SYNDROME IN YOUNG DAIRY CALVES: ROLE OF MYCOPLASMA BOVIS INFECTION

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Bovine respiratory disease is one of the most costly and widespread cause of economic loss in dairy farms, particularly as regards calves. Several microorganisms are involved in the pathogenesis of calf pneumonia and their role has been well defined by many authors. Among mycoplasmas causing respiratory disease, Mycoplasma bovis is considered the species most commonly associated with pneumonia in calves, where it can also cause arthritis, tenosynovitis and otitis. In northern italian regions, particularly Piedmont, Lombardy, Veneto and Emilia Romagna, to date little is known about the diffusion and epidemiology of Mycoplasma bovis infection among young dairy calves. In order to better evaluate the presence of this pathogen, during the years 2009 and 2010 a survey was carried out on calves carcasses sent to the Diagnostic Laboratory of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna in Brescia, regardless of the presence of specific lung lesions and the cause of death of the animals.

Samples were initially submitted to a PCR test for Mycoplasma spp. and positive specimens were tested with another specific PCR test for Mycoplasma bovis.

83 out of 224 (37%) lung tissue samples examined resulted positive at PCR test for Mycoplasma spp and in 64 cases we observed respiratory lesions (25 cases of chronic catarrhal bronchopneumonia, 3 cases of chronic bronchointerstitial pneumonia, and 36 cases of acute-subacute pneumonia). Mycoplasma bovis was identified in 26 out of 83 (31%) lung tissue samples positive at PCR test for Mycoplasma spp; in 24 cases of these we observed respiratory lesions (14 cases of chronic catarrhal bronchopneumonia, 1 case of chronic bronchointerstitial pneumonia and 9 cases of acute-subacute pneumonia).

Our data demonstrate how Mycoplasma bovis is an important respiratory pathogen in young dairy calves, so we think that further studies are needed to better evaluate the role of this pathogen in the respiratory diseases of young dairy calves; in particular, data about its real prevalence, its epidemiological features (including the role of apparently healthy subjects as carriers) and its real economic impact on farm management are needed.

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CORONARY ARTERY LESIONS IN THE SWORDFISH (XIPHIAS GLADIUS)

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Heart vessel lesions in the Swordfish have been investigated to highlight differences and similarities with the corresponding human and mammal alterations, and to better understand the histopathogenesis and the evolution of different vessel disorders.

Twelve hearts of Swordfish (Xiphias gladius) fished in the Ligurian Sea of Italy in 2012 were submitted to gross and histologic investigations, with particular attention to blood vessels, and stained with Haematoxylin-Eosin, Periodic Acid Schiff and Alcian blu. The age of the fishes ranged from few months to 10 years (average age 6 years).

At gross examination blood vessels of the atrium, ventriculum and bulb appeared normal, while histopathological evaluation of extramural and intramural coronary arteries showed different lesion classified as: intimal proliferation, modification in the inner elastic fiber and lesions of the tunica media.Intimal proliferation: both intramural and extramural coronary arteries showed intimal proliferations often associated with modifications of the inner elastic fibers. The severity of lesions varied from discrete protrusions to stenosis of the vessels. Modifications in the inner elastic fiber are the most frequent lesions in the artery of swordfish and show different degrees of severity, from partial to total thickening of the elastic membrane, duplication and fragmentation, thinning or loss of the elastic membrane.Lesions of the tunica media were observed only in the extramural arteries and are represented by proliferation of smooth muscles cells, partial or total loss of elastic fibers, extracellular vacuoles and reduction of smooth muscle cells; in one case an intimal fibrous proliferation and a necrotic area were detected. No correlation between lesions and their extension, animal age or gender have been observed.

Heart vessel lesions are well studied in mammals and humans, although their histopathogenesis and evolution have not been fully elucidated. Investigations in comparative pathology of the degenerative alterations of the coronary arteries are important tools to evaluate common factors responsible for the onset of those lesions in the different species.

CANCER MONITORING OF DOGS AND CATS LIVING IN A CHEMICALLY POLLUTED AREA: PRELIMINARY DATA.

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The presence of an important coal-fired power plant in the town of Quiliano, an area of the province of Savona, prompted us to design a monitoring activity to describe the geographical and temporal distribution of the malignant disease in resident pet animals (dogs and cats).
Aim of this work is to describe the cases collected during the first year of monitoring.

The Veterinary practitioners working in the area of interest including 16 municipalities have been met, made aware and invited to join a two-year program of pet cancer detection. Seven practitioners have collaborated over the first year of research by submitting, as prescribed, tissue samples of suspected neoplasms, fixed in 10% buffered formalin, accompanied by the clinical-anamnestic form. Reduction, inclusion, cutting and tissues staining as well as the histological diagnosis (1) and immunohistochemical investigations for diagnostic confirmation, were performed according to Standard Operating Procedures. All information collected were entered into a database for further statistical analysis.

In the first year of activity 48 samples (32 canine, 16 feline) were received from different municipalities of the area of interest: 4 cases were from Quiliano, town of the coal-fired power plant, and the others from the province of Savona. Seventeen canine tumours were benign (1 from Quiliano) and 11 malignant: 4 soft tissue sarcomas, 3 mammary gland carcinomas, 2 mastocitomas, 1 splenic hemangiosarcoma, 1 oral melanoma (from Quiliano). Feline samples included 2 benign tumors and 10 malignancies: 9 from skin/soft tissue (6 sarcomas and 3 carcinomas) and 1 oral squamous cell carcinoma; this last case as well as one of the sarcomas were from Quiliano. Eight cases were from non-neoplastic lesions.

These preliminary results are in line with what we previously observed in different geographical areas (2,3) without suggesting any special impact of the industrial plants of the area. Further work is needed to estimate the population denominator and the completeness of the incident tumors. Final results will allow a greater and better knowledge of animal tumor pathologies arising in this area, both for the comparison with those found in humans and to confirm or disprove possibly their relationship with any environmental determinants.

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CARDIAC LESIONS IN DOLPHINS STRANDED ALONG THE NORTHWESTERN ITALIAN COAST

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Little is known about the development and pathology affecting the cetacean heart. Most investigations are dealing with abnormal cardiac development in cetaceans. The aim of this study is to evaluate cardiac lesions in stranded dolphins.

Nine striped dolphins (Stenella coeruleoalba) and one male bottlenose dolphin (Tursiops truncatus) stranded along the Ligurian sea coast of Italy from February 2011 to April 2012 were examined. Age classes were determined, and field necropsies were performed. Hearts were submitted to histological examination and stained with Haematoxylin and Eosin (HE) and Weigert-Van Gieson stainings (WVG).

Gross cardiac changes were observed in 6 out of 10 animals, and histological lesions were found in 7 out of 10 dolphins. Gross evaluation of the hearts in three cases showed aneurysms of the pulmonary trunk. One dolphin had a dilated right ventricle, hypoplasia of the tricuspid chordae, severe and diffuse fibrosis associated with pronounced thickening and retraction of the tricuspid leaflets, and consequent left ventricle dilation. Adjacent to the papillary muscle in the interventricular septum, a focus of interstitial lymphocytic myocarditis was observed. Mitral valvular changes were observed in 3 cetaceans and included mitral fibrosis, mitral leaflet thickening, and left ventricular hypertrophy. In one case cirsoid aneurysm of the coronary arteries was observed. Histologically, the aneurysms of the pulmonary trunk showed a thinner wall compared to a normal one. WVG staining revealed thick, shattered and randomly arranged elastic fibers. In five animals histological examination of the mitral and tricuspid leaflets showed endocardiosis. Four dolphins showed also small pointed projections from the edges of the valve cusps identified as Lambl's excrescences.

Dolphin cardiac diseases show the same complexity of terrestrial mammals and humans. To the authors' best knowledge Lambl's excrescences, aneurysm of the pulmonary trunk, and cirsoid aneurysms have never been previously described in marine mammals, and some of these findings should be taken into account as a possible cause of dolphin strandings.

SURVEY ON TANK MILK CONTAMINATION BY MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN EMILIA-ROMAGNA REGION

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Mycobacterium avium subsp. paratuberculosis (Map), the causative agent of Paratuberculosis in ruminants, can lead to important economic losses in dairy herds. Moreover, Map has been evocated as cofactor in some human diseases with multifactorial etiopathogenesis(Crohn's disease and others). Milk can be contaminated both by direct excretion through the milk and by fecal contamination during milking of soiled udders (1).
Pasteurization has been shown to completely inactivate Map if the concentration in milk is not higher than 104-107/l (2).
Several extensive surveys have been carried out on bulk milk, but very few quantitative data are available (3); for this reason it is very difficult to estimate the exposure of consumers to Map contaminated milk.
For this purpose, we carried out a qualitative-quantitative survey on bulk tank milk (BTM) of all bovine dairy herds in Emilia-Romagna Region. During the period March-June 2013, 2788 samples were collected and analyzed.

Map detection on BTM was performed by a peptide-magnetic separation (PMS) protocol (4) followed by qPCR. Briefly, 50 ml of milk were centrifuged (2500 g for 15 min) and resuspended in 1 ml of PBS. Then, PMS capture of Map was done by addition of an equal volume of magnetic beads (Dynabeads MyOne Tosylactivated, Life Technologies) coated with peptides aMp3 and aMptD (Research Biochemicals). After capture, DNA extraction was performed by Chelex resin (Biorad). Quantitative PCR was done targeting IS900 and absolute quantification of Map cells was performed according to IS900-plasmid calibration [LOD calculated by IS900-plasmid dilution 1.5□1.0 x 101 bacteria/ml (5 replicates), LOD calculated by culture dilution 2.0□0.3 x 101].

Overall, 70 (2.55%) samples were positive by IS900 qPCR. Sixty-four (91.4%) of them were estimated to contain less than ten bacteria per ml of milk, while six (8.6%) showed a contamination ranging from 11 to 155 bacteria per ml. Interestingly, only two samples revealed a Map concentration between 73 and 155 bacteria per ml (IS900), confirmed by F57 qPCR (30 to 100 bacteria per ml of milk).

Our data suggest that Map contamination of bulk milk in dairy herds of Emilia Romagna Region is very limited; in fact only 0.2% of total samples showed high level of contamination (over 104 bacteria/l). Considering that low concentrations (<104/l) of Map can be inactivated by pasteurization, this survey suggests that the possibility of live Map being present in pasteurized milk from the dairy farms of Emilia-Romagna can be considered negligible.

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IMPEDANCE MEASUREMENTS FOR SALAMI PROCESS CONTROL. PRESENTATION OF THE "DRYCHECK" EU PROJECT

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In Europe salami have a long tradition originating from Mediterranean countries during Roman times. Then the production spread in Northern Europe, USA, Argentina and Australia; however, Europe is still the major producer and consumer of salami. There is a wide variety of salami on the European market as a consequence of variations in the raw materials, formulations and manufacturing processes, which come from the habits and customs of the different countries and regions.
In Europe the meat-based product processing, in particular the manufacture of salami, is mainly represented by small and medium enterprises (SMEs) which contribute substantially to the production and retail of those products. In most of the cases the SMEs involved in salami production are family related and frequently integrate with other sectors in the rural economy thereby enhancing local activities.
The challenge for SMEs producing salami lies in increasing production, minimizing product variability, assuring product safety and maintaining the typical sensory characteristics. Fermentation, drying and maturation are the main steps of salami process. The progress of these phases principally depends on the relative humidity and temperature inside drying and ripening chambers and is critical for product's final quality and safety.
In this context, the European Commission has funded the research project "Electrical impedance-based system to monitor and control the drying process in sausages" (DRYCHECK) within 7th Framework Program of the European Union, SP4 Capacities, Research for the benefit of specific groups, Research for small and medium enterprises.
The overall objective of DRYCHECK project is to provide SMEs a practical and non-invasive tool to improve the salami production reducing the variability of the products by a user-friendly, advanced and cost-efficient technology. The main goal of the project is to design and develop (a) a multi sensor system based on EIT (Electrical Impedance Tomography) able to monitor the drying/ripening process in representative European salami samples and (b) a drying chamber control system which uses the information from the EIT system, provided by wireless communication units based on ZigBee® technology, to readapt the environmental conditions in the chamber for a correct drying/ripening process.
DRYCHECK project is carried out by a consortium of 4 research and technological development centres (RTD performers) and 5 SMEs (www.drycheck.eu). .

INTESTINAL EMPHYSEMA IN NEBRODI BLACK PIGS: AN ABATTOIR SURVEY FROM 2003 TO 2013

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The aim of this study is to describe the intestinal emphysema cases observed in Nebrodi Black pigs of Sicily during the diagnostic activity from 2003 to 2013. Intestinal emphysema is an infrequent condition which affected swines. A similar pathology called Pneumatosis cystoides intestinalis is also recognized in man, and one case is reported in a rabbit. This condition is characterized by the presence of gas filled bubbles or thin-walled cystis within intestinal wall, mesenteric lymph nodes and mesentery. Although numerous explanations were advanced as to the cause of this condition (mechanical causes due to respiratory tract infections, nutrition, trauma, gastrointestinal infections and intestinal obstructions), actually the aetiology remains unknown.

From 2003 to 2013, during the abattoir surveillance, 15 of 3020 (0.5%) Nebrodi Black swine intestines showed macroscopic lesions compatible with intestinal emphysema. Gross and histological examinations of the involved tissues were performed.

Macroscopically intestine and mesentery contained diffuse vesicular lesions. Histologically several large endothelial-lined cystic structures surrounded by connective tissue within the submucosa and especially muscularis were detected. A mixed infiltration of inflammatory cells were also present in the connective tissue around the cysts.

Few data are available in the literature about this disease, however the incidence in pigs seems extremely low. Despite the low economic impact, the authors suggest further investigations about the aetiology and the possible consequences of this pathology on health, growth and wellness of the affected pigs.

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01 Sanità Animale e Sanità Pubblica

'CONTAMINATION' OF HORSE MEAT NOT DECLARED IN LABEL: PROFILES ONLY TRADE?

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Undeclared horsemeat in the label of industrial food. A question of a mistake in the label or a question of public ealth? We are talking about cases in Great Britain, in France and later in Italy about 'contamination' of horsemeat not declared on the label but found in frozen beef lasagne products of the same industrial food company. In UK and in France these products were retired by the authority; in Italy was the food company to withdrawl the products to avoid sanctions.
The aim of the present study is the analysis of the provisions on the traceability requirements of Food Law, the EC Regulation no. 504/2008. These documents regards the registry equine and italian laws concerning commercial aspects and public health. Above all is here analysed the ordinance of italian Health Ministry. The issue discussed is based on two points: is it a case of commercial fraud or is it a case of public health?

For the present paper we have used the reports published by the UK Government, the reports of the French Agriculture Minister, and Recommendation of the SCoFCAH. For Italy we have used the reports with the samples of the beef-products in question; samples ordered by the italian Health Ministry. The authors used the European Community and Italian regulations relating to the registry of equine, the official controls and Food Safety. The authors examined all the rules, identified the various powers of the authorities. they have analyzed the purpose of the regulations and their application.
RESULTS: The UK official reports say that the contamination of horsemeat undeclared on the label (detected in February 2013) is no warranty for traceability and does not indicate the duration of this trade. For these reasons it is not just a problem of commercial fraud but also a crime against public health.
The situation may suggest that there is a potential risk to the health of the consumer. English Reports state that the contamination is not an occasional error but was generated by a criminal conspiracy.
The SCoFCAH adopted a special plan to control the presence of horsemeat in products that do not declare on the label. This plan sets out a series of tests to detect the presence of phenylbutazone in horsemeat.
The italian Health Ministry, on a 361 samples of foods containing beef has detected that the presence of horsemeat is not declared on the label in 14 units.
Community and Italian laws devolved management of equine registry in the ministry of agriculture, in spite of the equine is considered food-producing animals.

The italian Health Ministry issued an Ordinance regarding registry equine health (1st march 2013). The document overrides the protection of public health and the health and welfare of animals; also orders the management of the Registry of equine animals, official controls and sanctions.

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DEVELOPMENT OF A CHECK-LIST FOR THE ASSESSMENT OF ANIMAL WELFARE DURING RITUAL SLAUGHTER IN THE PIEDMONT REGION: PRELIMINARY RESULTS

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The ritual slaughter has been always a much controversial and debated issue because in western society, welfare and protection of animals are very important values, shared by all. However, in most EU countries, for religious reasons, beef animals may be excluded from the stunning before bleeding. Obviously there are some difficulties due to this to ensure the protection of animal welfare. The aim of this work is to develop a survey depth of all slaughterhouses approved for religious slaughter of Piedmont, in order to acquire precise informations on the regional situation. This will allow all stakeholders to make use of a valuable scientific tool to assess the animals slaughtered welfare real conditions.

In the Piedmont Region there are 33 slaughterhouses in which you make slaughter according to religious rite and all these structure were involved during the study.

The result of the research was the development of a questionnaire with general information on the structure where the slaughter takes place, on practices applied to the slaughter, the kind of dedicated equipment and the destination of the meat, in order to describe the current situation to all evaluated slaughterhouses. In addition,thorough investigation was conducted by the official veterinarians of the slaughterhouse and were carried out site visits during slaughter operations that have enabled the development of a list of operational control that can be used on any type of animal that is slaughtered with ritual religious. In particular,the collection of information it is of fundamental importance to enable the data collection on the average time from the beginning of the restraint until the bleeding, the average number of cuts made, the average time for the debate, the average time of loss of rhythmic breathing, and other parameters that are thought to play a crucial role.

In recent years, the quantity of animals slaughtered according to the islamic rite, in Italy and in Europe, has increased significantly. However, an increasing consumption of meat "halal", has developed particular interest in the veterinarian officers and consumers, increasing conscious about welfare conditions of animal intended for human consumption. This preliminary work has allowed us to increase knowledge about the extent and implementation of religious slaughter in the Piedmont Region. Future goal will be to identify possible technical measures to limit the vigilance during slaughter operations. In addition, new protocols will be developed to improve both the principles of health and welfare, to prevent unnecessary suffering animals without infringing the fundamental principles of the Islamic religion.

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NATIONAL PROJECT – ITALIAN MINISTRY OF HEALTH EVALUATION OF THE TWO NATIONAL TYPES OF PIG SLAUGHTER AIMED TO THE MODERNIZATION OF MEAT INSPECTION

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The project is aimed to evaluate if the possible changes to EU regulations in swine inspection are compatible with the national swine production systems without increasing risks for consumers. This assessment will be performed also taking into account the hazards related to our breeding/production system which is composed of two different production systems.

As the national background is concerned, the national pig population is approximately 9.5 million. In particular, the pig production chain is characterized by the following data (Istat, 2010):
1. slaughtered pigs/year: 13,764,354 (national pigs plus pigs which come from foreign countries)
2. 64% of pigs (8,760,000 pigs) belongs to the integrated production system: "heavy swine line";
3. 36% of pigs of point 1 is slaughtered in low capacity production line plants;
4. about 7,500,000 pigs of point 2 (85% of 64%) are slaughtered in n.15 slaughterhouses which are located in 3 regions: Lombardia, Emilia Romagna and Piemonte.

In Italy, basically there are two types of swine production systems:
Type 1 - plants with high production capacity and Type 2 - plants with low production capacity.
The project plan will be organized in work-packages (WP) as follow:
WP 1 – study on the swine production chain (from farm to abattoir);
WP 2 – survey on food chain information (FCI);
WP 3 – analysis of the impact of changes on current practices of meat inspection as regard the two types of swine production systems.
The Parma O.U. activities will be carried out within Type 1 swine production system. In particular, at least 200,000 pigs will be monitored. Other activities of Parma O.U.:
- Set up of a platform data collection;
- Development of a software to collect data;
- Selection of slaughterhouses;
- Training of veterinary inspectors that will be involved in the project;
- Preparation of documents and set up of an informatic system
- Data and statistical analysis.

The Naples O.U. activities will be carried out within the Type 2 swine production system. In particular, not less than 8,000 pigs will be monitored. Other activities of O.U. Naples :
-Selection of slaughtering plants, identification and characterization of the farms of origin;
-Training of veterinary inspectors involved in the project;
-Random surveillance on-site on staff involved in the project and staff of the establishments;
-Control of recording, analysis and transmission of data to the coordinator of the project.

The Project is on-going

The Project is on-going

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OCCURRENCE OF AFLATOXIN M1 IN ORGANIC AND CONVENTIONAL MILK COMMERCIALIZED IN ITALY.

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Aflatoxins are the most toxic compounds produced by fungi, considered to be both genotoxic and carcinogenic (EFSA, 2007). The International Agency for Research on Cancer classified AFM1 as a class 2B possible human carcinogen (IARC, 1993). This experiment was established on the occasion of the special program of monitoring and surveillance of aflatoxin contamination, issued by the Ministry of Health at the end of summer 2012. The aim of the present study was to examine the occurrence of AFM1 in organic and conventional milk.

A total of 58 samples of milk brands commercialized in Italy were surveyed. The milk packages were purchased in markets and supermarkets between April and May 2013 as available to the final consumers. The samples included organic milk (n = 22), and conventional milk (n = 36). All the samples were analysed before their expiry date. Clean-up was done by using an immunoaffinity column (VICAM) and quantified by high-performance liquid chromatography with fluorescence detection.

The AFM1 calibration curve was linear in the concentration range considered, with a determination coefficient always > 0.999. Mean recovery from the milk spiked with AFM1 was 88%. No interfering substances were observed at the AFM1 retention time.
Aflatoxin M1 was detected in 60.3% of the samples, of which 18.9% organic and 41.4% conventional.
The levels of contamination were very low and ranged from 9 to 26 ppt, but only four samples showed a concentration of AFM1 greather than the limit of quantification (LOQ = 25ppt) whereas the other positives ranged between the limit of detection (LOD = 8ppt) and the limit of quantification.

Despite the high incidence of AFM1 found in the milk samples analysed, all samples complied with current EU regulations, which allow AFM1 content in milk up to 50 ng/L. Italia's monitoring and surveillance programs confirm the effectiveness of the regulatory controls in place for ensuring chemical residues in milk do not pose a threat to human health. In fact, a good food safety can only be ensured by continuous and numerous checks before commissioning the food trade.
AFM1 levels in organic and conventional milk samples did not present significant statistical differences, which is in accordance with the current bibliography.

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EVALUATION OF THE MICROBIOLOGICAL SCREENING TEST EFFICACY TO DETECT THE PRESENCE OF DRUG RESIDUES OF ANTI-BACTERIAL SUBSTANCES IN ANIMAL TISSUES AND FUTURE PERSPECTIVES.

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In order to protect public health, it is important to search drug residues in food of animal origin to find illegal animal treatments with forbidden drugs or improper treatments with permitted drugs.
At the moment, in Italy, routine laboratories do not perform multiclass chemical tests able to detect simultaneously different classes of drugs in animal tissues. The main reasons are related to the difficulties to develop methods for analytes with so large differences in the chemical structures and for the complexity of the tissues.
Therefore, to detect most of the veterinary drugs, a microbiological screening test has been performed: five different microorganism strains with different drug- sensitivity are used.
The aim of this work is to evaluate the efficacy of this test in the pharmacosurveillance; the data were collected over the last 5 years' activity of our Institute.

Petri plates, Brain heart Infusion, BHI agar, Antibiotic Medium 1 pH 6,5, Bacillus cereus ATCC 11778, Bacillus cereus K 250, Bacillus subtilis ATCC 6633, Escherichia coli 14, Kocuria rhizophila ATCC 9341, HPLC-DAD, microbiological screening test

On the basis of an accurate data evaluation it was observed that more than 70% of the muscle samples found positive using the microbiological test was confirmed by chemical methods.
The microbiological test, for example, is sensitive enough to detect Tetracycline residues; in fact, more than 80% of the suspected samples were confirmed, in some cases with values lower than 1/5 of the Maximum Residue Limit (100 ppb).
The microbiological test is also able to detect residues of Quinolones; their presence was confirmed in 65% of the muscle samples.
Instead, only 52% of suspected samples were confirmed for Beta-lactam antibiotics. One of the possible reasons may be that the chemical method is able to detect only Penicillins and not Cephalosporins.
The sensitivity of the microbiological test for Sulfonamides is inadequate; it detects only values much higher than MRL fixed at 100 ppb.
No data were available for Coccidiostats, because there were no confirmatory requests for these drugs during the last five years.

At the end of this study the evaluation of the microbiological test is satisfactory, only in terms of its simplicity, and for the fact that it is not expensive and allows routing tests in the shortest possible time.
Anyway, for the future it is important for the laboratory to develop sensitive multiclass chemical methods able to detect simultaneously a larger number of drugs used in livestock.

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SPECIES-SPECIFIC MONOCLONAL ENZYME-LINKED IMMUNOASSAY FOR DETECTION OF LUTEINIZING HORMONE IN BOVINE PLASMA

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The availability of reliable immunoassay for detection of luteinizing hormone (LH) is an existing problem and the development of new assays is subject for intensive studies in many species, including bovine (1). LH is a pituitary glycoprotein hormone consisting of two subunits, α and β, being the first in common with FSH and TSH. Due to the complex structure and the low plasma level, quantification of LH requires specific and sensitive assays. To date, bovine LH (bLH) is measured in a limited number of laboratories, often using reagents provided by external institutions.
Aim of the study was to develop a species-specific sandwich enzyme-linked immunoassay (ELISA) for quantification of bLH in plasma, using monoclonal antibodies (mAbs) and standard bLH both produced in our laboratory.

Hybridomas producing anti-bLH mAbs were obtained using USDA-bLH-B-6 as immunogen, and characterized as previously described (2). Anti-β-subunit mAb-N6H7 was used to purify bLH from pituitary extracts by immunoaffinity chromatography, and as capture mAb in the ELISA. Anti β-subunit mAb-N3G8 was labeled with biotin and used as second antibody in the ELISA. Purified bLH was characterized by SDS-PAGE, Western blot, amino acid analysis and nanoLC-ESI-MSMS, and the biological activity was measured in vitro with the MA-10 cell line (3).

The purification procedure yielded pure bLH, with specific activity of 2.1 U/mg, equal to reference USDA-bLH. In SDS-PAGE, pure bLH showed two bands (17400 Da and 18900 Da), corresponding to the α- and β-subunits.
mAbs N6H7 and N3G8, binding two non-overlapping epitopes on the β-subunit with high affinity (Kd 2.9 and 1.0 nM), were used to design a sandwich ELISA. N6H7 was immobilized on microtiter plates, and the bLH bound was revealed with biotinylated N3G8 and HRP-avidin. The ELISA showed a bLH standard curve linear over the range 0.05-2.5 ng/ml, and was suitable to measure bLH in plasma as shown by parallelism and recovery tests. Intra- and inter-assay CV ranged between 5.2%-9.6% and 11.5%-23.2%. No cross-reactivity (<1%) was observed with bFSH and bTSH.
The specificity of the ELISA was confirmed by GnRH stimulation test in heifers. Accordingly with other studies (4), circulating bLH concentrations slowly increased during the first hour, followed by a marked increase between 120 and 180 min after treatment.

We developed a new homologous, specific, sensitive and reproducible ELISA satisfying all the criteria required to investigate LH secretory patterns in the bovine and which may represent an effective tool to determine a wide range of physio- pathological conditions in cattle. Furthermore, the single step procedure developed to purify bLH and the use of mAbs ensure an easy source of ELISA reagents and long term continuity in operation of this assay.

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TREADMILL EXERCISE AFFECTS SEROTONIN TRANSPORTER EXPRESSION IN HORSE BLOOD PERIPHERAL CELLS

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Serotonin (5-HT) is a monoamine biosynthesized from L-tryptophan. Both 5-HT transport from blood into platelets/leukocytes and reuptake is mediated by a specific transporter SERT, characterized by 12-transmembrane domains (TM1-12) with six extracellular and five intracellular loops connecting each domain; a large extracellular loop between transmembrane domains TM3 and TM4 contains regulatory glycosylation sites; N- and C- ends are oriented towards intracellular environment. 5-HT uptake efficiency depends on plasma 5-HT level and number of transporters expressed on membrane. Once in intracellular environment, 5-HT is stored into dense granules and thrown into extracellular environment in response to biological factors (1,2). Experimental evidences suggest the key role of 5-HT in oxidative stress and, as well known, physical exercise is a powerful stimulator of reactive oxygen species (ROS) production (3). On this basis, we suggest an experimental protocol to highlight possible influence of treadmill exercise on 5-HT stockage in horse platelets and leukocytes by a SERT expression analysis. In addition, plasma 5-HT was measured through HPLC and cortisol by ELISA.

5 untrained horses (mean age 4±1) were exercised on a high speed treadmill (Mustang Kagra 2200) following a protocol of both walk (1.7 m/s, 5 min) and trot (3.4 m/s, 5 min) at 0% incline for a final 30 min workout. Blood samples in heparin were obtained both from pre- and post-exercise horses and immediately subjected to separation of leukocytes and platelets using density 1.077 g/mL sterile filtered ficoll. Once cells were separated, total RNA was extracted, quantified and retro transcribed to perform PCR Real Time reactions (ABI 7500). Two Assays on Demand (SERT and beta-actin) were used. Results were expressed according to ΔΔCt calculation and by a relative quantization software. 5-HT was detected by reverse phase HPLC and electrochemical detector (sensitivity 0.5 ng/ml), cortisol by ELISA Radim (sensitivity 5 ng/ml), both from a poor platelet plasma fraction. Statistics used Student's paired t-test.

After treadmill exercise a significant SERT mRNA decrease in both horse leukocytes and platelets and higher levels of plasma 5-HT and cortisol were detected.

A negative influence of treadmill exercise on SERT expression may be probably related to a minor 5-HT stockage in granules and consequent higher plasma levels. The increase of plasma cortisol agrees with stressful condition linked to exercise.

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HYPOALLERGENIC PROPERTIES OF DONKEY MILK

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Cow's milk protein allergy (CMPA) is an abnormal immunological reaction to cow milk proteins,IgE-mediated. The therapeutic strategy of CMPA is represented by the total elimination of milk or by the administration of cow's milk substitutes (1). Donkey's milk (DM) proved to be the best alternative in feeding infants affected by CMPA, since its chemical composition is comparable to human milk (1). In this work an "in vitro" study was performed in order to analyze the IgE reactivity to milk protein allergens from cow, donkey and goat by immunoblotting experiments using sera from milk-allergic and non allergic adult volunteers, with the aim of verifying the hypoallergenic property of donkey milk.

Blood samples were obtained from a total of 6 volunteers: 3 milk-allergic and 3 non allergic subjects. Milk caseins and whey proteins from cow, goat and donkey were separated by reversed-phase or anionic exchange chromatography. For the immunoblotting analysis, the purified proteins were firstly separated by SDS-PAGE and then transferred to a nitrocellulose membrane. The nitrocellulose sheet was subsequently incubated with each whole serum and therefore incubated with a mouse anti-human Ig-G secondary antibody.

The volunteer subjects involved in this screening were:
1A: subject affected by CMPA, 22 years old
2A: subject affected by CMPA, 25 years old
3A: subject with suspect CMPA, 25 years old
C1: control, 48 years old
C2: control, 46 years old
C3: control, 76 years old
The serum of the allergic subject 1A showed a weak cross-reactivity with bovine β-lactoglobulin, but a strong cross-reactivity was obtained towards the bovine □s1- and □s2- caseins. In addition, the 1A subject showed a cross-reactivity also with goat □s2-casein. No positivity was observed in correspondence of the DM proteins. The serum of allergic subject 2A showed no cross reactivity with any of the blotted whey proteins, indicating that its allergy is probably only due to casein fraction. Subject 2A showed in fact a very strong cross-reactivity towards all the bovine caseins fraction, cross-reacted also with the goat □s2-casein but showed no cross-reactivity with the DM proteins. The serum of subject 3A with suspected CMPA did not show any cross-reactivity with any of the blotted whey proteins, while a weak cross-reactivity towards the bovine casein fractions was observed, but not towards the goat and the DM proteins. The control subjects C1, C2 and C3 did not shows cross-reactivity with any of the blotted proteins.

This study provided a preliminary prove on the hypoallergenicity of DM if compared to bovine and goat milk. However, due to the limited number of cases examined, further studies are needed to confirm these data.

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PROTEOMIC ANALYSIS OF MULTI DRUG RESISTANCE IN STREPTOCOCCUS UBERIS: A PRELIMINARY STUDY

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Streptococcus uberis is an opportunistic bacterium, responsible of a significant percentage of both clinical and subclinical cases of bovine mastitis. It is an environmental bacterium but characterized by high penetrability and persistence within animal tissues. This is mainly due to the adaptability of S. uberis to different “niches” (pasture, water, litter, nipple, glandular tissue, milk etc.) and the ability in switching their metabolism to generate resistance to several classes of antibiotics (Egan, 2012). The aim of this study was to assess the molecular mechanism of multidrug resistance in S. uberis isolated from bovine mastitic milk by using a proteomic gel-based approach and combined MS procedures.

A multi drug resistance (R) and one drug-susceptible S.uberis strains, isolated from bovine mastitis, were used in this study. Proteins were obtained after cell rupture by sonication, removal of cell debris by centrifugation and clean up by precipitation. The two-dimensional electrophoresis was performed on IPG strip pH 4-7 and on SDS-page 12% T. The comparative densitometric analysis of the stained gels were performed by using the PDQuest 2-D analysis software (Bio-Rad). Spots whose staining intensity was significantly different in the two groups with a fold change ≥ 2 or ≤0.5 were further considered for MS identification by using nanoLC-ESI-LIT-MS/MS analysis. Blast2GO websites were used to Gene ontology and functional annotation analysis of the identified proteins (Chiaradia, 2013).

Comparative 2-DE analysis of proteins profile from drug resistance and drug-susceptible strain revealed 42 protein spots showing a significant quantitative difference ($P<0.05$). These spots were submitted to analysis for protein identification, which led to the final recognition of 31 deregulated protein entries. Gene ontology analysis indicated that most of the deregulated proteins take part in the primary metabolic process, in cellular carbohydrate metabolic process, metabolism of nitrogen compound, nucleobase-containing metabolic compound, gene expression, generation of precursor metabolites and energy, protein metabolic process. KEGG analysis suggests that S. uberis counteract the antibiotic action modulating in particular the purine, fructose /mannose , and pentose metabolism as well as the glycolysis/gluconeogenesis.

Proteins and molecular pathways identified after proper validation, could contribute to a better definition of the metabolic peculiarities of S. uberis and the identification of molecular targets for the development of new therapeutic strategies.

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LEPTIN AND ITS RECEPTOR IN WILD AND DOMESTIC SWINE CARPAL GLANDS

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Pig carpal glands are skin glands specialized in the production of a volatile substance involved in the transmission of scent information and are located in the subcutaneous layer of the carpus caudomedial surface.
Their histological and histochemical features have been extensively investigated in both domestic and wild pigs [1, 2 and 3] and have provided important information regarding secretion chemical characteristics. The aim of the present work is to highlight the presence of leptin and its receptor in these glands and to verify the possibility of difference expressions between wild and domestic subjects, with the pourpose of providing data useful to better understand the mechanisms regulating their functionality.

Eight clinically healthy adult male pigs, four domestic and four wild, were used. Carpal gland specimens were obtained immediately after slaughter, fixed in 4% formaldehyde solution and subsequently processed for embedding in paraffin, following routine tissue preparation procedures.
The immunohistochemical reaction was visualized, utilising the avidin-biotin-complex and the DAB as the chromogen. The following primary antibodies were used: anti-Ob rabbit polyclonal antibody and anti-Ob-R goat polyclonal antibody.

The immunohistochemical study showed a strong positivity for leptin and its receptor in the carpal glands of the animals examined. In particular, the immuno-reaction seems to affect only the cytoplasm of the dark cells while the clear ones are always negative. Immuno-positivity for leptin and its receptor was not observed in the epithelium of the ducts or in the connective tissue. In addition, there were no appreciable differences between domestic and wild swine.

Recent studies conducted on some exocrine glands have allowed researchers to highlight how the same are able to secrete leptin and may represent a target of the action of this protein [4,5 and 6]. This has enabled them to understand how the action of the Ob protein is much more extensive than first imagined. In this sense the results obtained in the present study confirm what has previously been stated and allow us to hypothesize that this gland is able to produce leptin and is itself a target of this molecule that may act by in autocrine/paracrine manner without being influenced by the different conditions of life.

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LOCALIZATION OF LEPTIN RECEPTOR (OB-R) IN THE SKIN OF RUMINANTS: AN IMMUNOHISTOCHEMICAL STUDY.

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Leptin receptor (Ob-R) is the product of the diabetes (db) gene and is a member of the class I cytokine receptor superfamily (1). It is highly expressed in the hypothalamus although its expression has also been demonstrated in many peripheral tissues. Leptin is a polypeptide mainly secreted by adipose tissue, regulating appetite and energy consumption (2). However Ob-R distribution suggests that leptin might exert diverse biological functions other than energy metabolism regulation.
Accordingly, leptin acts as a mitogen for a large number of cell types, including the epidermis where it has been detected together with its receptor. In particular, leptin strongly stimulates a proliferative response of keratinocytes during skin repair (3). Leptin and its receptor have also been detected in human and mouse hair follicle (HF) and an involvement of leptin in the control of HF morphogenesis has been suggested (4, 5). The aim of this work is to study the presence of Ob-R and to localize it in the skin of ruminants.

Skin samples, collected from the dorsal region of healthy animals, were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Dewaxed sections were microwaved in 10 mM citric acid (pH 6.0) for antigen retrieval and endogenous peroxidase activity was quenched with a peroxidase-blocking solution (3% H2O2). Subsequently, the slides were incubated with 1:100 goat polyclonal anti Ob-R antibody (Santa Cruz Biotechnology) for 24 hours. After incubation with 1:200 horse anti-goat biotin conjugate antibody (Santa Cruz Biotechnology), the reaction was visualized with the Vectastain ABC kit and revealed with DAB.

The immunohistochemical investigation allowed us to evidence a clear positivity of Ob-R in some components of ovine and bovine skin. In both species, cytoplasmic staining was observed in the cells of the outer root sheath of the HF at the level of the isthmus and the soprabulbar region. The signal was present in both growing and regressive phase HF. As regards sheep, Ob-R positive cells were also observed in the basal layer of the epidermis.

At present, leptin is considered one of the major players in the biology and pathology of mammalian skin and its appendages. In humans, it represents a novel therapeutic factor to improve severely disturbed wound-healing conditions. The identification of Ob-R in the skin of ruminants represents an important goal to understand the biological mechanisms involving this molecule with implications in the clinical field.

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ALPHA-TRANSDUCIN EXPRESSION IN THE GASTROINTESTINAL TRACT OF THE EUROPEAN SEA BASS (DICENTRARCHUS LABRAX)

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The gustatory system plays the critical function of distinguishing between nutrients and non-nutrient, potentially dangerous substances (1) allowing adaptation to different habitats (2). Different tastes are detected by G protein couple receptors and their signalling molecules,including the heterotrimeric G proteins,α-transducin (Gαtran)and α-gustducin(Gαgust) (3,4). Taste-related molecules have been discovered in the gastrointestinal tract of a variety of species from fish to humans. In this study we examined the distribution and peptide content of cells expressing Gαtran-immunoreactivity in the gastrointestinal(GI)mucosa of the sea bass.

Adult European sea bass(Dicentrarchus labrax)were sampled from different tanks and euthanized by an overdose of anaesthetic. The stomach,pyloric caeca and intestine were harvested;the intestine was divided into three regions: cranial, middle and caudal. Tissue sections were processed for immunofluorescence with the following primary antisera: Gαtran, Gαgust, ghrelin(GHR), 5-hydroxytryptamine (5-HT), obestatin (OB), somatostatin (SOM), gastrin/cholecystokinin (GAS/CCK), glucagon-like peptide-1 (GLP-1), calcitonin gene-related peptide (CGRP) and substance P(SP). Each antibody was either used alone for single labelling or in combination with others for double labelling.

Gαtran immunoreactivity was observed throughout the sea bass GI tract, but was more abundant in the stomach compared to the intestine with decreasing density of cells from the cranial to the caudal regions. Specificity of Gαtran and Gαgust immunostaining was established by Western blot analysis, which showed immunopositive bands at the expected molecular weight of ~45 and ~40 kDa, respectively, in sea bass gut tissue as well as in positive tissue (e.g. brain and eye). Staining specificity was also demonstrated by immunoblocking with the homologous peptides. Colocalization of Gαtran and Gαgust immunoreactivity was visualized in some cells in the stomach. A subpopulation of Gαtran immunoreacitve cells in the stomach also contained GHR, OB or 5-HT immunoreactivity. Colocalization of Gαtran immumoreactivity with SOM, GAS/CCK, GLP-1, SP, or CGRP immunistaining was not observed in any GI tract regions.

Our data provide evidence that Gαtran and Gαgust are involved in chemosensory transmission in the sea bass GI enteroendocrine system. It is likely that nutrients activate taste receptors and their signalling molecules to induce the release of peptides from enteroendocrine cells, which in turn activate other cells types directly or via neural reflexes thus initiating a variety of physiological responses controlling GI functions and caloric intake.

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DISTRIBUTION OF PAR-2 IMMUNOREACTIVITY IN THE HORSE INTESTINE

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Proteinase-activated receptors (PARs) are a family of G-protein-coupled receptors which are uniquely activated through the proteolytic cleavage of their N-terminal domain. Four members of the PAR family have been cloned: PAR-1, PAR-2, PAR-3 and PAR-4. PARs are expressed in various organs, including the gastrointestinal tract (Soh et al., 2010). The PAR-2 is widely distributed in rodents and human gastrointestinal tracts where it is involved in regulating mucus and pepsin production, gastrointestinal motility, intestine ion transport, gut permeability, and acute inflammatory response (Kawabata et al., 2008). Despite the previous studies, no information is known on the distribution of PAR-2 in the gut of the horse. Thus, in the present study, immunohistochemistry techniques were used to determine the cellular localization of the PAR-2 in the jejunum, ileum and colon (pelvic flexure) of the horse.

Pieces of jejunum, ileum and colon were obtained at the public slaughterhouse from 8 horses. The tissues were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose solution, frozen in isopentane cooled in liquid nitrogen, and sectioned at 12 µm on a cryostat. Cryostat sections were processed for immunoperoxidase and immunofluorescence.

The PAR-2-immunoreactivity was observed in the enterocytes (apical and basolateral surfaces), intestinal glands, and interstitial cells of Cajal-like elements. However, PAR-2 immunostaining was also observed in some neurons, immune cells, and smooth muscle of the muscularis mucosae and muscularis externa. Interestingly, there was no difference in the distribution of PAR-2 immunoreactivity in the different intestinal tracts.

In enterocytes, PAR-2 can be activated by luminal as well as circulating proteinases. Apical stimulation of PAR-2 appears to increase paracellular permeability (Bueno and Fioramonti, 2008). In contrast, basolateral PAR-2 activation could induce neutrally independent Cl- secretion (Kawabata et al., 2008). The presence of the PAR-2 in the intestinal glands indicates that this receptor could regulate glandular exocrine secretion as observed in parotid, sublingual and lacrimal glands (Kawabata et al., 2008). PAR-2 plays a role in modulation of motility of the intestinal smooth muscle. Primarily, PAR-2 located in smooth muscle cells mediates directly the contractile activity. However, also the stimulation of PAR-2 located on both enteric neurons and interstitial cells of Cajal could modulate the gut contractial activity. Finally disorder associated with intestinal inflammation could be associated with the activation of PAR-2. In fact, as observed in rodents and humans (Vergnolle, 2008), also in the horse PAR-2 could be expressed in several inflammatory cells, such as neutrophils and mast cells.

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SENSORY INNERVATION OF THE PORCINE URETHRAL MUSCLE

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The urethral muscle (UM) is a pelvic striated muscle involved in the voluntary control of micturition, that needs complex interactions between autonomic and somatic afferent and efferent pathways. The afferent fibres travelling in the pudendal nerves are both myelinated and unmyelinated and can modulate bladder activity being sensitive to urine flow, contraction of the muscle itself and painful stimuli (1-2). For a better understanding of visceral nociception and excretory mechanisms we studied the site, morphology and neurochemical characteristics of primary sensory neurons projecting to the UM of the pig, considered an important animal model for biomedical studies.

After injection of 50 µl of Fast Blue (FB) in the ventral side of the UM of two 40-Kg boars, the bilateral T14-Ca1 spinal ganglia (SG) were collected, cryosectioned and immunohistochemically processed to assess the co-existence of the vanilloid receptor (VR1) with substance P (SP) and neuronal nitric oxide-synthase (nNOS) within FB positive (FB+) neurons.

We observed a thousand FB+ neurons bilaterally in S2-S4 SG. Their area ranged from 300 to 2000 µm2. The majority (~69%) of the FB+ cells tested was VR1-immunoreactive (VR1-IR). Part of them (~12%) was also nNOS-IR, while only ~4% was also SP-IR. nNOS-IR and SP-IR neurons were found in ~16% and ~6% of the FB+ neurons tested.

The presence of FB+ neurons almost exclusively in the S2-S4 suggests that afferent fibres from the pig UM travel in the pudendal nerves.
The VR1-IR small/medium sized neurons could be the source of the unmyelinated nociceptive fibres responding to noxious chemical and mechanical stimuli, already documented in the lower urinary tract (3). Activation of these afferents triggers painful sensations as well as body defence mechanisms such as inflammation and bladder hyperactivity that eliminate infectious or irritating, potentially injurious, agents from the urinary tract.
nNOS-IR neurons were observed in a small number of cells, but it is known that their number increases as a consequence of pathological lesions. They synthesize NO, a gaseous neurotransmitter that should act in a retrograde manner both in SG and in the spinal cord modulating multisynaptic local circuits that process nociceptive inputs (4).
SP-IR neurons were scarce and most of them co-expressed VR1. They could trigger central autonomic reflex and peripheral axon reflexes which modulate smooth muscle activity and facilitate transmission in autonomic ganglia in an anterograde manner.
In conclusion, our results indicate an important role of nociception among afferent inputs from UM that facilitate the micturition reflex and promote complete bladder emptying.

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MACROSCOPIC ANATOMICAL STUDY OF THE SPIRAL LOOP OF THE ASCENDING COLON IN CATTLE FETUSES

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The spiral loop of the ascending colon (SLAC) in cattle has the form of a flattened disk consisting of two centripetal coils, a central loop and two centrifugal coils. It is attached to the great mesentery and it is located, with its longitudinal major axis, in the middle of the mass of the jejunum. In the last few years during autopsies or surgery to resolve pathologies such as volvulus, intussusception, and intestinal torsion, the frequent occurrence of abnormalities of location of the spiral loops (with or without detachment from the mesentery) was evidenced. So much so as to advance the hypothesis that these abnormalities might facilitate the above mentioned pathologies (Rademacher and Gentile, 2008). Moreover, a research on slaughtered calves showed a prevalence of SLAC abnormalities in 42.4% of the animals (472/1,113) (Gentile et al.; 2013). The deviations range from a simple reduction of the adherence to the mesentery ("conical relaxation", with spiral coils loosely attached to the mesentery. 381/1,113 = 34.2% in the above mentioned slaughtered calves) to a situation of a partial detachment ("partial dystopia", with distancing of only some spiral loops form the mesentery. 68/1,113 = 6.1%, see above). The worst case is the complete dislocation of all the spiral loops from the mesentery disk ("ectopia", with detachment and total movement of the entire elliptic disk form its central mesentery position. 23/1,113 = 2.1%, see above). Aim of the present study was to verify whether abnormalities of location of the SLAC can be found also in the prenatal life.

A macroscopic examination of the SLAC of 58 Holstein bovine fetuses was carried out. Fetuses size ranged from 14 cm to 80 cm in crown-rump length (CRL).

All together 23 out of 58 fetuses showed a conformation of the SLAC different from what expected on the base of anatomical texts. In particular, 10/58 (17.2%) had a conical relaxation, 5/58 (8.6%) a partial dystopia and 2/58 (3.4%) a complete ectopia. Furthermore, SLAC of 6/58 fetuses (10.3%) , although having a correct adhesion to the great mesentery, showed abnormal spiraling. Finally, in 16/58 fetuses (27.6%) the spiral loops, instead of having two centripetal coils and two centrifugal coils, as normally described in veterinary anatomy texts, demonstrated just one and a half centripetal coils and one and a half centrifugal coils.

This study supports the hypothesis that the abnormalities encountered previously in adult animals are, for the majority, of a congenital nature. The high prevalence of a conformation of the SLAC different from what described in anatomy texts, at least for the lightest deviations, indicates the need of reconsidering the true normal anatomical condition of the SLAC.

Rademacher and Gentile Tieraerztl. Umschau, 63:133-141 (2008); Gentile et al. Buiatria 8:51-62 (2013).

ANATOMICAL STUDY OF SOME MUSCLES OF THE SHOULDER, ARM AND FOREARM IN THREE SPECIES OF WILD BIRDS

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In the past many authors (Baumel et al., 1993; George et al., 1966; King et al., 1985) have focused on the anatomical study of the wing in order to correlate anatomical details with the peculiarities of flight in different species.

In spite of the limited information about the anatomy of the thoracic limb in European avian species, we decided to investigate the related muscles in three species presenting a different kind of flight spread throughout the Italian territory: the Grey Heron (Ardea cinerea), the Eurasian Buzzard (Buteo buteo) and the Common Kestrel (Falco tinnunculus). Therefore we performed a stratigraphic dissection of the wing in different subjects of the species examined.

Of all the muscles that were examined, for the sake of brevity, only the musculi deltoideus and flexor carpi ulnaris will be considered. These muscles demonstrated, in fact, the most interesting characteristics.
Regarding the m. deltoideus, the part that demonstrated the most peculiarities was the p. propatagialis. The species most in line with what is described in anatomical literature was the Eurasian Buzzard. In the Grey Heron the propatagial ligament in its central part commences a fibrous fascia which inserts into the m. extensor carpi radialis and which ends in a single fascia on the ventral side of the carpometacarpal bone. In the Common Kestrel it is interesting to note how the propatagial ligament presents, not only a ventral termination, but also a fibrous fascia that goes to the dorsal side of the distal extremity of the radius.
In the Eurasian Buzzard and in the Grey Heron the m. flexor carpi ulnaris at its proximal end displayed the presence of a fibrous thickening similar to a sesamoid. To the contrary, in the Common Kestrel no fibrous thickening was observed at the proximal end. In this species, instead, the fleshy component is more developed.

Regarding the p. propatagialis of the m. deltoideus, that which was observed in the Grey Heron we believe can contribute to maintain the propatagial tension. In this way vibrations of this structure, which could cause diminished lift, are avoided. The peculiarity evidenced in the distal insertion of the Common Kestrel could instead influence the control of the pronation-supination of the hand during kiting.
Relative to the m. flexor carpi ulnaris, we believe the presence of a sesamoid-like structure at the base tendon, found in the Grey Heron and in the Eurasian Buzzard, may have the purpose to complete the articular surfaces of the elbow. In this way the dislocation of this joint is avoided and this insertion is reinforced rendering it more resistant to tensile stresses.

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FUNCTIONAL ROLE OF MUSCARINIC RECEPTORS IN THE CONTRACTIONS OF HORSE ISOLATED BRONCHI

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Acetylcholine (ACh) is the main neurotransmitter which induces bronchoconstriction in horses and other species and has a paramount role in the pathogenesis of obstructive respiratory diseases [1]. However, the knowledge about the functional role of ACh muscarinic receptors in the regulation of horse airway contractions is limited.

Bronchial smooth muscle rings were obtained from lungs of slaughtered horses, put in isolated organ baths and connected to isotonic transducers which measured the length modifications of the preparations. The effects of different concentrations (10-8-10-5 M) of nonselective (atropine) and selective muscarinic M1 (VU0255035), M2 (methoctramine), M3 (pFHHSiD) receptor antagonists on the contractions evoked by electrical field stimulation (EFS) and exogenous ACh were evaluated. EFS was applied by means of two platinum electrodes delivering to the tissue trains of square wave pulses (1 ms, 50 mV, 50 Hz) every 120 s. Regular phasic contractions were obtained, and the percentage of variation of pre-drug amplitude of contractions induced by the antagonists was measured. In the second set of experiments, concentration-response curves of ACh were constructed before and 30 min after each antagonist was added into the organ bath solution. In separate experiments, the effects of muscarinic antagonists used in combination (at 10-6 M) on EFS-induced contractions were evaluated. The potency of each antagonist in EFS experiments was measured by the concentration giving 50% of maximum effect (EC50) from individual concentration-response curves and expressed as pKi value (-Log EC50). Antagonist potency at muscarinic receptors in the experiments with exogenous ACh was expressed with pA2 value, calculated by Gaddum's equation.

EFS-induced contractions were of cholinergic nature, since atropine was able to inhibit them dose-dependently, reaching a maximal effect of 85.8% at 10-6 M. Conversely, VU0255035 was ineffective, while methoctramine and pFHHSiD reduced the contractions in a considerable degree only at the highest concentration (10-5 M). The experiments with exogenous ACh confirmed that atropine was significantly more potent than selective antagonists in inhibiting bronchial contractions. The simultaneous block of M1/M3 or M2/M3 receptors, obtained with the antagonists in combination, inhibited EFS-induced contractions with an efficacy comparable to that of atropine; M1/M2 blockade was instead ineffective.

Even though M3 receptors have a central role in the cholinergic contractions of horse bronchi, both M1 and M2 subtypes seem to possess a cooperative function. Selective muscarinic antagonists do not represent a valid alternative to common nonselective drugs, whereas an anticholinergic with M1/M3 affinity could be an excellent bronchodilator without the side-effects due to M2 block.

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EFFECTS OF SHOW JUMPING COMPETITION PLUS TRANSPORT STRESS ON TOTAL AND FREE IODOTHYRONINE CHANGES OF SPORT HORSES

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Thyroid hormones are considered as a marker of stress in sport horses, showing changes in according to training, exercise and transport. The aim of research was to studied the total and free iodothyronine changes of No. 6 jumping horses (7-12 years old), performed in show jumping competition, after competition, after pre-competition and post-competition transport, and after competition plus transport.

Blood samples were collected in basal conditions, before and after transport, before exercise, and 5 and 30 minutes after exercise. Serum total and free iodothyronine concentrations were analysed using commercial immunoenzymatic assays (EIA, RADIM, Rome, Italy). A 1-way repeated measure analysis of variance was applied to test the effects of competition, transport and competition plus transport on basal values.

Compared to basal values, no significant changes were observed for total and free iodothyronines both after pre-competition, post-competition transport and post-competition plus transport. Post-competition transport basal T3 values were higher ($P<0.01$) than pre-competition transport basal values, and post-competition transport basal fT4 values were lower ($P<0.01$) than pre-competition transport basal values. A significant effect of competition on fT3 changes ($F=4.227$; $P<0.05$) was recorded. Competition plus transport basal T3 values were higher ($P<0.001$) than competition basal values, while competition plus transport basal fT3 values were lower ($P<0.001$) than competition basal values.

Results obtained showed that transport and show jumping competition differently influenced the thyroid responses of trained sport horses to physiological and psychological stress, also on dependence of jumpers' recovery period and individual performance. The increase of total and free iodothyronines after transport confirmed previous data observed in sport horses after short road transport, showing that T3 represents the hormonal metabolic active form in response to stressful stimuli (1,2). The lowest basal fT4 concentration after competition plus transport showed the effect of additional physical effort and that the recovery period was probably inadequate. The highest basal T3 and the lowest fT3 values of horses submitted to transport plus exercise showed their primary involvement in maintaining functional homeostasis after competitive exercise. In addition, no significant changes of total and free iodothyronines 5 and 30 min after competition confirmed previous data observed in experienced jumpers submitted to transport before exercise.

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EFFECT OF RESVERATROL ON LIQUID STORAGE OF STALLION SEMEN

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Resveratrol is a natural polyphenol with antioxidant and/or pro-oxidant proprieties depending on its concentration. In order to improve the quality of stored sperm, supplementation of mammalian semen with Resveratrol has been tested in few studies (Collodel et al., 2011, Silva et al., 2012).
The aim of this work was to evaluate the effect of Resveratrol during stallion sperm liquid storage.

Fresh ejaculates collected from two stallions were mixed and samples were diluted with Kenney extender to 30×106 spermatozoa/ml. Aliquots of 2ml of diluted semen were stored at either +4 or +10°C, in absence (CTR group) or presence of different Resveratrol concentrations (10, 20,40, 80 µM).
Viability(SYBRgreen14/PI staining), acrosomal integrity (FITC-PSA staining), mitochondrial membrane potential (JC1/SYBRgreen14/PI staining), motility (CASA system) and chromatin integrity (SCSA) of spermatozoa were assessed at 0 h and after 24 h storage.
Data were analyzed by ANOVA followed by the Tukey post hoc test for multiple comparison. Data are expressed as mean percentage ± standard deviation.

Resveratrol did not induce any effect on either overall viability or total motility (TM); however, a significant decrease ($p < 0.01$) of viability, TM and progressive motility (PM) in 24h stored semen compared with fresh one, independently from Resveratrol supplementation and storage temperature, was observed.
A significant ($p < 0.01$) reduction of overall percentage of spermatozoa with PM was recorded in all groups supplemented with 40 and 80 µM Resveratrol.
The incidence of acrosome-intact spermatozoa significantly ($p < 0.01$) decreased after 24 h storage, independently from storage temperature.
A significant ($p < 0.05$) decrease of viable spermatozoa with high mitochondrial membrane potential (SYBR+/JC1+/PI-) was evident after 24 h storage in 40 and 80 µM Resveratrol groups.
This negative effect was also recorded comparing, 40 and 80 µM Resveratrol groups with CTR group after 24 h storage.
Sperm DNA integrity was not affected by storage time, storage temperature or Resveratrol treatment.

In conclusion, our findings demonstrate that, at the tested concentrations, Resveratrol supplementation does not enhance sperm quality of stallion semen after 24h of storage. Moreover, 40 and 80µM Resveratrol concentrations could damage sperm functional status, probably acting as pro-oxidant.Finally, although 24 h of storage significantly affect the majority of sperm quality parameters, no significant differences were found in groups maintained at +4 or +10° C, suggesting that stallion semen could be equally preserved at these different temperatures.

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EFFECTS OF TRAINING SESSIONS ON BETA-ENDORPHIN, LACTATE AND HEART RATE CHANGES OF SPORT HORSES BEFORE AND AFTER SHOW JUMPING

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β-endorphin, blood lactate and heart rate (HR) changes of sport horses are considered as markers of stress and welfare, training state and vegetative responses. The aim of this research was to investigate the effects of training sessions on circulating β-endorphin, blood lactate and HR changes in sport horses before and after show jumping and their correlations.

No. 8 trained jumpers (6 geldings and 2 females, 7-12 years old), performed in four consecutive training sessions before and after competition; the circuit design, intensity and duration of training sessions were the same before and after competitions. Blood samples were collected from the jugular vein, in basal conditions, before training sessions, and after training sessions. The HR was obtained before the blood sampling. Plasma β-endorphin concentrations were measured using a commercial radioimmunoassay kit (Peninsula Lab., Inc., Belmont, CA). Blood lactate concentrations were measured using Accusport tester.

β-endorphin, lactate and HR showed higher values after training sessions than basal values both before and after competition. Compared to basal values, β-endorphin ($P<0.001$), lactate ($P<0.05$) and HR ($P<0.05$) showed higher values after training sessions before competition; β-endorphin values showed no significant increase, while higher lactate ($P<0.05$) and HR ($P<0.01$) values after training sessions after competition were recorded; 2-way RM-ANOVA showed that fence height did not significantly affect β-endorphin, lactate and HR changes. No significant differences between basal values of β-endorphin, lactate and HR before and after competition were observed. No significant correlations among β-endorphin, blood lactate and HR, and between these variables and different fence heights were observed.

The increase of β-endorphin after training sessions both before and after competition confirmed its involvement in stressful conditions with a high emotional content, in accordance with previous data reported after competitive, sub- and maximal exercise (1,2); in addition, this trend reflected the need for modulation of fatigue and pain perception even in trained horses, although independently of difficulty level. The absence of a significant correlation between β-endorphin changes and different fence heights confirms previous studies reported after show jumping competitions and after experimental show jumping (2). The highest lactate and HR values after the training sessions showed the effect of additional physical effort involved during the four consecutive training sessions observed before and after competition.

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EFFECT OF ALKALINE PHOSPHATASE ON IN VITRO FERTILIZATION OF PORCINE OOCYTES

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Alkaline phosphatase (AP) is diffused in several body tissues and recently has it also been demonstrated to be involved in the regulation of boar sperm capacitation (Bucci et al. , 2012).
The aim of the present study was to examine the effect of AP on in vitro fertilization in swine species.

Pools of 2 ejaculates from 5 different boars were used and at least 5 repetitions were performed. Boar sperm samples were collected and washed twice with PBS supplemented with 0.4% BSA and finally resuspended to 5x105 sperm/ml in capacitation medium (Brackett & Oliphant's (BO) + 12% foetal calf serum + 10µg/mL progesterone) . Aliquots of 500µl of sperm suspensions were incubated in Nunc 4-well multidish for 1 h at 39°C 5% CO2, 7% O2 in presence of different doses of AP: 0, 0.6, 1.2, 2.5 IU (CTR, AP 0.6, AP 1.2 and AP 2.5, respectively). At the end of the capacitating incubation, 50 IVM oocytes were added to each well. After 1.5 h of gamete co-culture, oocytes were transferred to fresh IVF medium and cultured for 19 h until fixation.
The effect of AP on fertilization was assessed by evaluating the number of fertilized oocytes (penetrated oocytes/total inseminated) and monospermy (number of oocytes containing only one sperm head or male pronucleus/total fertilized).

The results showed a significant decrease in the percentage of fertilized oocytes in response to increasing AP doses, starting from AP 1.2. In fact, while the same percentage was observed in control and AP 0.6 group (72.1±2.6 % and 75±11% respectively), a significant ($p<0.05$) decrease was recorded both in AP 1.2 (47.1±13.5%) and AP 2.5 (17.8±2.7%). A parallel increase in monospermic zygote rate was observed (60%, 70%, 80% and 100% in CTR, AP 0.6, AP 1.2, AP 2.5 respectively; $p<0.05$).

Our results demonstrate that AP inhibits in vitro fertilization of porcine oocytes probably by acting on spermatozoa in a dose dependent manner; our previous results (Bucci et al., 2012), in fact, showed an inhibition of boar sperm capacitation exerted by AP. Furthermore, a high activity of the enzyme in seminal plasma together with the decrease of its activity in sperm extracts after capacitation let us to suppose that AP could be involved in maintaining spermatozoa quiescent until fertilization.

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REGULATION OF TASTE SIGNALING MOLECULES BY HIGH PROTEIN DIET IN THE PIG PYLORUS

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Taste receptors (TRs) and their signaling molecules are widely expressed in extra-oral sites, including the gastrointestinal (GI) tract mucosa(1). Fasting and refeeding have been shown to modify the expression of α-transducin / α-gustducin in enteroendocrine cells of the pig GI tract(2), however, the effects of individual nutrients on TR-related molecules remain unknown. The gustatory system is fundamental for detecting dietary nutrients and evoking appropriate functional responses leading to digestion and absorption. Thus, the aim of this study was to test whether a short- and long-term high protein diet (3 and 30 days, respectively) affected the enteroendocrine profile of the TRs signalling molecules α-transducin / α-gustducin expressing cells in the pig gastric mucosa.

Twelve hybrid (LW x D) female pigs (weighing 30-40 Kg) were subdivided in three experimental groups (n= 4 each group): A) fed standard diet (controls, CTR, 14,5% protein); B) fed high protein diet for 3 days (HP-3, 33% protein); and C) fed high protein diet for 30 days (HP-30, 33% protein). The protein used to supplement the diet was fish and potatoes protein. Mucosal samples from pylorus were harvested, fixed and processed for single and double labelling immunofluorescence with a mixture of the following primary antisera to Gα-transducin (Gαtran), Gα-gustducin (Gαgust) and 5-hydroxytryptamine (5-HT).

In the pyloric mucosa, the average number of Gαtran immunoreactive (-IR) cells were 111.5 ± 26.2 (CTR), 148.8 ± 13.2 (HP3), and 218.3 ± 28.8 (HP30) (CTR vs HP-3 $P< 0.04$; CTR vs HP-30 $P< 0.002$; HP-3 vs HP-30 $P< 0.005$), while Gαgust-IR cells were 138.8 ± 45.1 8 (CTR), 177.3 ± 14 (HP3), 211.5 ± 29.1 (HP30) (CTR vs HP-30 $P <0.004$, HP-3 vs HP30 $P <0.001$). The average number of Gαtran/5-HT-IR cells was 107.8 ± 28.6 (CTR), 141.3 ± 14.2 (HP3) and 207.5 ± 22.8 (HP-30) (CTR vs HP-3 $P < 0.002$, HP-3 vs HP-30 $P < 0.003$), while the Gαgust/5-HT-IR cells were 127.3 ± 44.3 (CTR), 161 ± 13 (HP3) and 203.3 ± 24.8 (HP-30) (CTR vs HP-3 $P <0.002$, HP-3 vs HP30 $P <0.003$).

High protein diet regulates the expression of Gαtran / Gαgust in the 5-HT containing enteroendocrine cells in the mucosa of the pig pylorus, supporting a functional role of taste related molecules in chemosensing in the GI tract.

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2)Mazzoni M et al.(2013) J Cell Mol Med 17(4):466-74.

IDENTIFICATION OF THE TRUNCATED FORM OF FELINE RON/STK IN FELINE MAMMARY CARCINOMAS.

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RON/stk is a member of the MET receptor family, over-expressed in human breast cancer and identified in cat as feline-stk receptor. The human RON gene codifies the full length and the short length transcripts, encoding for the full-size and the truncated form of the receptor respectively. The truncated form is generated from an alternative transcriptional start from a second promoter within the intron 10 of RON. The short form of RON lacks the N-terminus of the protein including most of the extracellular domain but conserve the kinase activity of the COOH terminus after heterodimerization. In human the short form of RON plays an important role in breast cancer and its expression is correlated to invasive capability in vitro.
The aim of this research was to evaluate the expression of full length RON and its truncated form in feline mammary carcinomas (FMC) samples and cell lines.

Immunohistochemical expression of RON was evaluated on 46 paraffin embedded FMCs tissues after incubating with polyclonal antibody anti RON (Sc-260 Santa Crutz) at diluition 1:150. Total protein from 6 FMC cell lines were extracted and after immunoprecipitation with RON antibody linked to sepharose G protein, the proteins were subjected to western blot and incubated with RON polyclonal antibody. To detect the truncated form of feline RON, RT-PCR with primers annealing on exon 10 and exon 11 was performed on cDNA of 6 FMC cell lines and 11 FMC.

the 78.26% of FMCs analyzed expressed RON receptor although any statistical correlation with clinical follow-up and RON expression was found. The truncated form of feline RON was detected in 4/6 FMC cell lines and in 6/11 feline carcinoma tissues. The genomic sequence of introns 10 and 11 was determined.

This is the first evidence of the presence of truncated form of RON in feline tumors suggesting that also in FMC it can be involved in tumoral progression as well in other feline tumors. As demonstrated in human, we found that also in feline RON is highly expressed in FMC tissues suggesting its role in tumor progression and representing a suitable target for therapy with RON inhibitors.

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Xiaoliang Ren et al.APMIS 2011
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CHOROID PLEXUS TUMORS IN DOGS: HISTOPATHOLOGICAL STUDY AND CLASSIFICATION

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Choroid plexus tumors (CPTs) are rare intracranial neoplasms arising from intraventricular choroid plexus. In the recent years they have been reported with an increasing incidence in dogs (6,9%), probably due to the progressive improvement in veterinary diagnostic imaging (1). Currently two major grades of CPTs are recognized in domestic animals, Choroid Plexus Papilloma (CPP) and Choroid Plexus Carcinoma (CPC) (2), while in human WHO histological classification three grades are recognized (CPP, atypical CPP, CPC). The aims of this study are to verify the applicability of human histological grading system to canine species, and to compare the results of this study with previous reports, in order to contribute to the knowledge enrichment about the biological behavior of canine CPTs.

Paraffin embedded tissue from 15 selected canine CPTs was included in the present retrospective study. 5 µm sections were stained with H&E. The selected CPTs were graded according to the criteria of the latest human WHO international histological classification of CNS tumors. Diagnostic criteria for CPPs were histological features of normal choroid plexus, with or without minimal local brain invasion, and < 2 mitotic figures per 10HPF. Histological criterium for atypical CPPs was ≥ 2 mitoses per 10HPF, and up to 2 of the following features were also considered: increased cellularity, nuclear atypia, loss of papillary pattern, and areas of necrosis. Criteria for CPCs included at least 4 of the following histological features: ≥ 5 mitotic figures per 10 HPF, nuclear atypia, multilayering of the epithelium, increased cell density, loss of papillary pattern with solid cell sheets and multifocal areas of necrosis. The proliferative index of these tumors was assessed by immunohistochemistry (ABC, Dakocytomation, Denmark) using anti-Ki-67 antibody (gene expression MIB-1, 1:10, Santa Cruz Biotechnologies, Europe).

Based on human WHO classification system, 6 tumors (40%) were classified as CPP, 7 tumors (46,6%) were classified as aCPP, and the remaining 2 tumors (13,4%) as CPC. Immunolabeling for Ki-67 showed evidence of increasing immunoreaction in relation with histological grade.

In this study an intermediate histological grade (aCPP, grade II) between CPPs and CPCs has been identified in dogs for the first time. Intraventricular spread was observed in low grade tumors, confirming that in dogs as in humans the ability to metastasize within the ventricular system and the subarachnoid space cannot be consider alone as a sign of CPC. However, further studies are necessary in dogs to attribute the exact biological behavior to grade I and grade II CPTs and to verify if, as in humans, their ability in producing drop metastases can compromise post-surgery survival rate.

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EXPRESSION PROFILE OF MUC1 IN CANINE MAMMARY TUMOUR

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Mucin1 is encoded by MUC1 gene and anchored to the apical side of epithelial cells by a transmembrane domain. In woman, MUC1 overexpression is related to aggressive behaviour and poor outcome of neoplasms(Devine et al. 2009). In particular, in carcinoma cells with loss of polarity, MUC1 is expressed at high levels over their entire surface and, moreover, it is aberrantly expressed in most tumours diagnosed each year in the USA. MUC1 overexpression is one of the most common alterations in human cancers(Andrianifahanana et al., 2006, De Oliveira et al., 2009,Gum et al., 1992, Kufe et al.,2009).
The aim of our study was to analyse the expression profile of MUC1 in canine mammary tumours to verify if it can be used as a diagnostic and prognostic marker.

Eight bitches were subjected to mastectomy at the Faculty of Veterinary Medicine in Grugliasco.Three were affected by simple carcinoma, three by complex carcinoma and two had a benign tumour.For every subject, two samples of mammary tissue were obtained: one from the tumoral tissue and another from the surrounding mammary tissue. The samples were immediately put into O.C.T. and stored at -80°C.The samples were homogenized, then RNA was extracted, purified and eluted. RNA was reverse-transcribed to obtain cDNA which was amplified through qRT-PCR. Three housekeeping genes were used to correct the variability between samples.

Our results indicate that in bitch MUC1 is expressed both in tumour and in healthy tissue. In simple carcinoma, the gene is overexpressed in tumour compared with healthy tissue, whereas in complex carcinoma it is downregulated in tumour compared with healthy tissue. In benign tumours, the level of expression is the same as in the surrounding tissue; therefore this level of expression was used as a reference value. Compared to this value, in simple carcinoma MUC1 is overexpressed from 7 to 35 times in tumour and from 0 to 17 times in the surrounding tissue, whereas in complex carcinoma MUC1 is overexpressed from 5 to 12 times in tumour and from 10 to 76 times in healthy tissue.

This preliminary study, which should be continued increasing the number of individual samples, offers a first contribution to the study of the expression profile of MUC1 in mammary canine carcinomas through qRT-PCR. MUC1 results to be expressed both in neoplastic and morphologically not neoplastic tissue. A possible explanation of this phenomenon could be the fact that the activation of the gene precedes the neoplastic transformation of tissue. Moreover, the transcript of MUC1 is present at higher levels in carcinomas than in benign tumours.

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PRELIMINARY RESULTS ON SKIN INCISION, TISSUE DAMAGE AND WOUND HEALING ON IN-VIVO PORCINE SKIN MODEL: A COMPARISON BETWEEN DIFFERENT INCISION METHODS, BASED ON SURGICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL RESULTS. PART ONE: SURGICAL EVALUATIONS

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Aim of this study was to compare quality of incisions and lateral thermal injury (LTI) produced by Diode Laser (DL), CO2 Laser (CO2L), Thulium Laser (TL), monopolar electrosurgery (MES) and Steel Scalpel (SS) and their effects on the reepithelialisation of incisional wounds of porcine skin

Eighteen, 10-14 week old, mixed breed (large white x landrace) pigs were employed in this preliminary experimental study. In each animal 3 standard 6 cm length full-thickness skin incisions were performed at a distance of 6 cm apart from one another, in the lateral portion of the left flank. Incisions were carried out using the following devices: DL (808 nm, 600 nm optical fibre, continuous mode, average power 5 W), CO2L (10600 nm CW and Pulsed mode, average power 6 W e 10 W), TL (2013 nm CW mode, average power 26 W), MES (cutting mode, power 40 W) and SS (n. 10 blade) as a gold standard cutting instrument. Each incision was scored (scale of 0 to 5) for: execution time, cutting precision, degree of bleeding and degree of cauterization. The Kruskal Wallis test was applied to compare groups. A P value of <0.001 was considered significant. In order to evaluate the histological and immunohistochemical results, full-thickness specimens were harvested using a 8 mm dermal punch on days 0 (end of incisional procedure), 4 and 12. To minimally affect the wound healing, skin suture was accomplished by staplers (35 W) and specimens on day 0, 4 and 12 were obtained respectively on the proximal, middle and distal part of each incision. At the end of the experimental study (60 days) pigs were sacrificed and the entire incisions were harvested for further evaluations.

Quality of each incision was evaluated macroscopically using the following parameters: Execution time: DL and TL required significantly more time in order to perform a complete incision compared with the other tested instruments.Precision of cutting: SS was the most precise overall. CO2L and MES were significantly more precise than DL and TL.The latter two devices were characterized by a wide variability between the observations.Degree of bleeding: SS presented the highest degree of bleeding and the scores were statistically greater in comparison with the other devices. No significant differences were noted among the other devices. Degree of cauterisation: CO2L produced a significantly superior degree of cauterization compared to the other devices, while MES, DL and TL were significantly superior to SS that achieved the lowest score overall.

From a surgical standpoint the best surgical incisional quality in the porcine skin model was obtained using CO2. This device was characterized by both cutting precision as well as rapid execution time; furthermore it produced optimal results in terms of cauterisation and bleeding control.

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PRELIMINARY RESULTS ON SKIN INCISION, TISSUE DAMAGE AND WOUND HEALING ON IN-VIVO PORCINE SKIN MODEL: A COMPARISON BETWEEN DIFFERENT METHODS, BASED ON SURGICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL RESULTS. PART TWO: HISTOLOGY AND IMMUNOHISTOCHEMISTRY

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The device used for skin incision can deeply affect final results and patient's recovery time. Lateral thermal injury (LTI) and processes of wound healing after incision are pivotal in the patient's outcome1. Aims of this study are to compare incisional damage and lateral thermal injury (LTI) produced by Diode Laser (DL), CO2 Laser (CO2L), Thulium Laser (TL)2, monopolar electrosurgery (MES) and Steel Scalpel (SS) on porcine skin incisional wounds. Moreover, it aims to get deeper insights on any differences on wound healing processes.

Skin biopsies from eight 10-14 week old, mixed breed (Large White x Landrace) pigs were collected at day 0, 4 and 12 from surgical wounds induced with different devices. Eight-mm biopsy punch samples were formalin-fixed, paraffin-embedded and routinely stained (H&E, PAS, Masson's trichrome). The following parameters were evaluated: LTI, epithelial and BM damage, depth of injury, vascular damage, inflammation, fibroblast reactivity, granulation and scar tissue. Subsequently, immunohistochemistry was performed to evaluate Ki-67 index of epithelial cells on the incision margins.

SS incision was considered the reference model. Bioptic samples from TL incisions showed severe LTI, with a mean extension of 1080,7 µ. DL produced less extensive LTI (mean 625,3 µ), while MES and CO2L incisions were characterized by similar LTI values, comprised between 200 and 325 µ. Dermal immature scar tissue on day 12 was prominent on samples from TL incision, where it exceeded the limits of the biopsy. Also DL and MES healing incisions were characterized by abundant scar tissue, whereas in samples from CO2L incision it was less extensive, even with milder fibroblast activation compared to SS. Notably, in all specimens where adipose tissue was damaged by incision, a prominent fibrous panniculitis was noticed. Higher Ki-67 indexes were observed at day 4, particularly in samples with most severe LTI recorded on day 0. Ki-67 index was clearly decreased at day 12. Massive inflammation was observed in specimens where infection was evidenced with intraoperatory bacteriological test.

Therefore, this preliminary study evaluated damages produced by different surgical devices and their outcome in different phases of wound healing. Result from our data indicate CO2L as a valid device to perform skin incision on porcine model, whereas TL and DL did not appear suitable devices. Anyway, further studies are needed to support this hypothesis.

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MOLECULAR EVOLUTION OF PSITTACINE BEAK AND FEATHER DISEASE VIRUS (PBFDV) ISOLATED FROM AFRICAN GREY PARROTS (PSITTACUS ERITHACUS) REARED IN ITALY

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The purpose of this study was to molecularly characterize BFDV strains isolated in Italy from African grey parrots affected by peracute or chronic form of BFDV disease. To our knowledge this is the first survey of BFDV infection in Italy in which, together with the classical sequence comparison analyses, we attempted to associate genetic features of the viruses with clinical forms of the illness.

Mixed organs from 8 animals dead displaying peracute form of BFDV disease and blood from 2 animals harbouring chronic signs of this illness were collected. These animals were reared in 8 different breeding facilities located in North and Central Italy.
The full-length genomes of BFDV strains were analyzed using 4 primer sets that amplify overlapping DNA stretches. These fragments were sequenced and aligned with all known BFDV sequences available on GenBank using the program ClustalW (1). Bayesian methods implemented in the computer program MrBayes ver. 3.1.1 (2), were used to draw phylogenetic trees and assess statistical support for clades.

All genomes contained the characteristic nonanucleotide circovirus origin of replication sequence located within a stem loop structure and the conserved motifs located within the 2 major open reading frames (ORFs). A peculiar feature of the BFDV viruses we analyzed was the presence of 5 to 6 ORFs, in contrast with published data that ascribed to BFDV genome the presence up to 7 ORFs. ORF6 was always absent.
The whole nucleotide sequence identity among our isolates varied from 93.6% to 99.9% and genome sizes extended from 1,997 to 2,001 nucleotides.
Full genome analysis showed that the DNA isolated from 8 animals fall into 2 subtypes of the BFDV-J strain, namely J1 and J2, which share a 98 to 100% intra-subtype identity, while the sequences of BFDV viruses obtained from other 2 animals, based on the classification system proposed by Varsani et al. (2011), seem to cluster into 2 new subtypes, which we defined as J4 and J5. Finally, by phylogenetic analysis, we observed a correlation among viral strains derived from the same breeding, whereas no association between phylogenetic distribution and clinical symptoms or geographical location of the breeding was noticed.

Our study shows that the complete genomes of BFDV strains isolates in Italy grouped into BFDV-J strain, according to the fact that this is the main strain infecting African grey parrots in Europe. Moreover, even if we were not able to discern an association between genetic feature of the virus and form of the illness, we identify two new circovirus subtypes, namely J4 and J5 characteristic of Italian BFDV viruses.

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THE EFFECTS OF SOME ANESTHETIC AGENTS ON MICROCIRCULATION EVALUATED BY LASER DOPPLER PERFUSION IMAGING (LDPI) IN MICE.

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Anesthetics can alter microvascular perfusion, affecting tissue oxygenation and delivery of vital substrates. The α2-adrenergic agonist dexmedetomidine and the α-blocker acepromazine are powerful sedatives with remarkable hemodynamic effects. Some authors reported an attenuation of the α2-adrenergic agonist pressor response by an acepromazine-xylazine combination in dogs. We investigated non-invasively the microcirculatory effects of dexmedetomidine, of acepromazine and of their combination in isoflurane anesthetized mice by Laser Doppler Perfusion Imaging (LDPI).

Thirty-two age-matched and sex-paired CD1 mice underwent 1.5% isoflurane anesthesia, followed by intraperitoneal injection of either 5 mg/kg acepromazine, or 1 mg/kg dexmedetomidine, or by their combination. Body temperature was adjusted to 36 °C. Heart (HR) and breath (BR) rate were recorded. Hind paws blood flow (Perfusion Units, volt) was recorded by LDPI 10 and 20 minutes after isoflurane induction, at different intervals after treatments, and after reversing dexmedetomidine by the α2-antagonist atipamezole.

BR decreased in all groups without significant differences to baseline ($P>0.05$). Dexmedetomidine sharply reduced over time HR ($P<0.001$), while atipamezole gradually reported HR close to baseline ($P>0.05$). Acepromazine+dexmedetomidine decreased HR ($P<0.001$), reaching steady values after 5 minutes ($P>0.05$); atipamezole gradually raised HR close to baseline ($P>0.05$). Peripheral perfusion under isoflurane anesthesia showed an increasing trend after 10 and 20 minutes, without differences among groups ($P=0.1$). Acepromazine increased perfusion between 10 and 20 minutes ($P=0.005$). Dexmedetomidine reduced blood perfusion after 5 minutes ($P=0.0001$), followed by an increase after 15 minutes ($P=0.008$). No significant changes were seen 5 minutes after atipamezole ($P=0.9$). Acepromazine+dexmedetomidine resulted in steady perfusion values over time ($P=0.44$), which after atipamezole increased very close to baseline ($P=0.237$).

Acepromazine+dexmedetomidine in mice produced more temperate, steady peripheral perfusion values compared to those following single agent, reducing the entity of the α2 -agonist biphasic hemodynamic pattern. Our translational approach by LDPI in a mouse model allows an easy, accurate and non invasive measurement of the effects of anesthetics on peripheral microcirculation.

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Groups	*10 min*	*20 min*	*25 min*	*30 min*	*35 min*	*40 min*
1	4.37/3.73-4.90	4.57/3.67-5.52		4.53/3.98-5.53		4.84/4.29-5.76
2	4.32/3.69-4.75	4.52/3.62-5.33	2.37/2.02-3.13		4.32/3.73-4.81	4.41/3.72-4.72
3	4.35/3.70-4.87	4.55/3.64-5.47	3.58/2.23-4.63	3.77/2.92-4.65	3.85/2.94-4.63	3.80/3.14-4.55

Mice peripheral PU (volt) of isoflurane+acepromazine (1), isoflurane+dexmedetomidine (2), and isoflurane+ acepromazine + dexmedetomidine (3) experimental groups at different time points (median/min-max).

GROWTH PATTERNS AND BODY COMPOSITION EVALUATION IN C57BL/6J MOUSE STRAIN BY DUAL ENERGY X-RAY ABSORPTIOMETRY.

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Growth curve models are routinely used in biomedical research to better understand overall development of the body and its components, and the pathogenetic mechanisms underlying growth and metabolism disorders. Wild type or genetically engineered C57BL/6J mouse strain is widely used in a great variety of research fields on growth and metabolism. The objectives of this study were to investigate, in a noninvasive and longitudinal way, the growth pattern and changes in body composition in this mouse strain.

Body weight, surface area and composition were obtained from four (n. 20) eight, twelve, sixteen and twenty weeks aged female mice. Relative rate of growth between 4 vs 8 and 8 vs 20 weeks of age were defined as :RG= 100[(W2- W1)/ (t2-t1)]/ [(W2- W1)/ 2]. Dual energy x-ray absorptiometry (DEXA) imaging was used to determine in vivo lean, fat, bone mineral concentration (grams) and density (grams/cm2) over time. Body lean, fat and mineral bone content values were normalized for body surface area (BS, in m2), according to the DuBois equation: BS (m2)=0.007184 x body weight (kg) 0.425x body length (cm) 0.725 1, to obtain the correct projected areal bone mineral density (BMD g/cm2 * m), lean mass index (LMI) and fat mass index (FMI).

Body weight and surface area increased linearly from four to twenty weeks of age (weight range: 16,1 ± 0,6 – 23,4 ± 0,8 g; surface area interval: 51,3 ± 0,07 – 65,4 ± 0,09 cm2). Rate of growth increased by 50% between 4 and 8 weeks old mice, whereas grew only the 16,5% from 8 to 20 weeks of age. Bone mineral density peak was reached at sixteen weeks of age (0,68 ± 0,02 g/cm2 * m). Lean and fat mass showed inversely correlated changes describing a sigmoid curve, with an evident gain in body protein at eight (0, 278 ± 0,01 g/ cm2) and twelve weeks (0,281 ± 0,01 g/ cm2) of age, and a slow prevalence of fat mass increase from twelve (0,043 ± 0,001 g/ cm2) to twenty (0,060 ± 0,0015g/ cm2) weeks of age.

Dual energy X-ray absorptiometry (DEXA) has been utilized in a variety of clinical and research applications in the fields of nutrition, metabolism and bone physiology. This study was designed to form the baseline for future comparison of wild-type animals with those in which altered skeletal growth, body composition and metabolism have been induced by surgical, pharmacological or genetic manipulation. DEXA appears a useful technique that permits longitudinal measurements of body composition, according to the principle of “refinement, reduction, and replacement”, and improving the accuracy of experimental results.

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Age, weeks	No.	Body Weight, g	Body surface area, cm²	BMD corr, g/cm² * m	LMI [1], g/cm²	FMI [2], g/cm²
4	20	16,1 ± 0,6	51,13 ± 0,0007	0,53 ± 0,05	0,24 ± 0,01	0,042 ± 0,005
8	20	20 ± 0,9	56,66 ± 0,0009	0,61 ± 0,02	0,27 ± 0,01	0,037 ± 0,003
12	5	21,2 ± 0,8	60,2 ± 0,0008	0,64 ± 0,01	0,28 ± 0,01	0,043 ± 0,001
16	5	22,4 ± 0,5	63,2 ± 0,0001	0,68 ± 0,02	0,27 ± 0,01	0,057 ± 0,001
20	5	23,4 ± 0,8	65,4 ± 0,0008	0,63 ± 0,01	0,27 ± 0,008	0,060 ± 0,005

Mean and standard deviation values for body weight, surface area, correct projected areal bone mineral density, lean and fat mass index in female C57BL/6J mice from 4 to 20 weeks of age.

[1] LMI = lean mass index

[2] FMI = fat mass index

EVALUATION OF ANTIOXIDANT CAPACITY IN SEMINAL PLASMA AND SPERM CELL EXTRACT OF BOAR, RAM, AND GOAT

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Cold shock can injure spermatozoa in different levels and can alter spermatozoa functional integrity hence reducing their fertilizing ability. A significant reduction in the level of spermatozoa antioxidants has been reported as one of the causes of the enhanced susceptibility of these cells to peroxidative injuries after cryopreservation. The aim of this work was to investigate whether there are specie-specific differences in the total antioxidant capacity and total thiols and GSH content in seminal plasma and sperm cell extract among boar, goat and ram.

Samples were processed rapidly and kept frozen at − 80 °C until assayed. TAC of seminal plasma and sperm cell extract was determined using the TEAC assay as described by Re et al. (1999). Briefly, the TEAC assay measures the relative ability of circulating antioxidants to scavenge 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cations (ABTS+) in comparison with the antioxidant capacity of Trolox standards. The absorbance was measured at 734 nm and the antioxidant activity was expressed as Trolox equivalent. GSH and total thiols content were determined in sperm cell extract by spectrophotometric method using dithionitrobenzoic acid (DTNB) by Ellman's assay as described by Patsoukis and Georgiou (2004).

Obtained results showed that GSH and total thiols content were significantly lower in swine compared to small ruminants ($p<0.01$). On the other hand, both seminal plasma and sperm cell extract showed a significantly higher TAC in swine compared to small ruminants. Within the same species, seminal plasma proved to have a higher TAC compared to sperm cell extract ($p<0.05$).

This results suggest that boar spermatozoa and seminal plasma may be endorsed with higher enzymatic antioxidant defense or with non-enzymatic compounds other than thiols, such as alpha-tocopherol, beta-carotene, ascorbate, or urate. Further studies are needed to characterize the concentration of the full spectrum of possible anti-oxidants in these three species. This finding can be applied to develop protocol to optimize semen cryopreservation protocols, but may also have an application in developing therapeutic protocols for male infertility related to a decrease in sperm antioxidant defense system.

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FIRST DETECTION OF CTX-M PRODUCING ESCHERICHIA COLI ST131 IN CATS WITH URINARY INFECTION IN ITALY

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The objective of this study was to screen a group of E. coli isolated from cats with urinary tract infection for the presence of blaCTX-M genes

Fifteen E. coli isolates were collected in 2012 from feline patients affected by cystitis. All strains were screened for blaCTX-M gene by PCR (1) and 7 samples were positive. These E. coli were tested to investigate other β-lactamase genes: blaTEM, blaSHV and blaCMY-2 (2). Amplicons positive for blaCTX-M, blaTEM and blaSHV were sequenced to identify the variants. They were analyzed by Repetitive Extragenic Palindromic (REP)-PCR typing and Multilocus Sequence Typing (MLST) and tested by the disk diffusion method to determine antimicrobial resistance. The following antibiotics were used: amikacin (AK), amoxicillin (A), amoxicillin-clavulanate (AC), ceftriaxone (CTR), cefepime (FEP), chloramphenicol (CLR), enrofloxacin (E), gentamicin (G), imipenem (I), nalidixic acid (NA), nitrofurantoin (N), spectinomycin (S), streptomycin (STR), sulphonamide (SUL), tetracycline (TE), trimethoprim (TRIM).

Seven of the 15 strains showed the presence of blaCTX-M gene, in particular 5 strains were CTX-M-14, one was CTX-M-1 and one CTX-M-15. Two strains contained also blaTEM-1 and blaCMY-2. We identified 4 distinct STs by MLST analysis: ST131, ST555, ST602 and ST155. REP-PCR generated a pattern consisting of two distinct groups plus the strain ST131, that was genetically distinct from the others. All CTX-M E. coli were not susceptible to the β-lactams (except carbapenems) and exhibited a phenotype of multiresistance. All isolates were susceptible to imipenem.

The identification of 7 E.coli blaCTX-M (47% of samples) provides evidence for diffusion of these genes within the Italian feline population. In particular we found the epidemiological clone ST131, a virulent strain spreading worldwide, frequently described as urinary pathogen in humans (3, 4) but rarely isolated in companion animals (5, 6). The study provides the first report of uropathogenic E. coli ST131 isolate in cats in Italy. Furthermore, we detected the presence of ST602, recently described in avian species in Italy (7).

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Table. Characterization of 7 CTX-M producing *E. coli* isolated from feline urinary samples

Isolate	REP-profile	MLST	Type of ESBL	non-β-lactam resistance phenotype
Cat 1	Group 1	ST555	CTX-M-14	S, STR, G, AK, TE, NA, CLR
Cat2	Group 1	N.D.*	CTX-M-14	S, STR, NA, E, CLR
Cat 3	Group 1	ST155	CTX-M-1	S, STR, G, SUL, TRIM, N, TE, NA, ENO
Cat 4	Group 2	ST602	CTX-M-14	S, STR, TE, NA, E, CLR
Cat 5	Group 2	N.D	CTX-M-14+ CMY_2	S, STR, G, SUL, TRIM, TE, NA, E
Cat 6	Group 3	ST131	CTX-M-15	S, STR, G, SUL, TRIM, NA, E
Cat 7	Group 2	N.D.	CTX-M-14+ CMY_2+TEM1	S, STR, G, SUL, TRIM, TE, NA, E

*ND: not determined

GENOTYPING THE SWINE HISTOCOMPATIBILITY ANTIGENS (SLA) BY DIRECT SEQUENCING: METHOD VALIDATION AND DETECTION OF NOVEL ALLELES

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Several colonies of pigs are used for research in medicine (NIH miniature swine, Sinclair, etc.) because different breed of pigs may be more appropriate in different fields in research, and also it is possible to diversify animals. Numerous inbreeding between animals, in fact, leads to a homogeneity of genetic traits, while for special issues such as transplantation, where the histocompatibility is critical, it is important to have the largest number of possible combinations. The effective genotyping of the swine major histocompatibility antigens (SLA) is essential for establishing a colony of SLA-defined pigs (1, 2).
The aim of our study is to develop and validate an accurate and cost-effective molecular screening for genotyping type I and type II SLA loci, having these loci a main role in the transplantation compatibility.

DNA was extracted from whole blood by Blood purification kit using Maxwell 16 System (Promega Corp). PCR and sequencing primers were designed in conserved regions of exon 3 of SLA1 and DQB1 genes using Primer3 software (http:/frodo.wi.mit.edu). Amplified fragments were sequenced using BigDye Terminator sequencing kit and 3730 DNA Analyzer (ABI). Sequences were aligned to reference alleles available on IPD-MHC Database (www.ebi.ac.uk/ipd/mhc/sla/) by Sequencer software (Gene Codes Corp.). The protocol was developed and validated on 8 pigs, then additional 6 Swine Pathogen Free (SPF) pigs were properly selected and genotyped to carry out inbreeding for obtaining a colony of SLA-homozygous pigs (genetically pure pigs).

Five out of six SPF pigs resulted SLA-identical, showing heterozygosity for two novel alleles at SLA1 locus and homozygosity for the known c.DQB1*0801 allele. The alleles segregation in the offspring will be analyzed to detect the genetic phase and to attribute the nomenclature at the novel alleles. The genetically different pig resulted homozygous for a novel allele at SLA1 gene and for the known c.DQB1*0101 allele. As the novel SLA1 allele showed high similarity with SLA1*11 allele, the provisional name we suggest is SLA1*11aa01.

Our sequencing protocol for SLA genotyping resulted accurate, cost-effective and powerful, allowing to easily identify also previously undescribed alleles.

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ANTIBACTERIAL ACTIVITY OF HONEYS PRODUCED IN PIEDMONT AGAINST PATHOGENIC BACTERIA IN COMPARISON TO MANUKA HONEY

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The antimicrobial activity of honey has been recognized for a long time, but with the increase of antibiotics, medical use of honey is significantly diminished. In recent years the rise of multiresistant bacteria caused the return to alternative methods. The purpose of the study was to evaluate in vitro antimicrobial activity of different commercial honeys of local production (Piedmont) for a possible use in the medical and surgical fields.

A selection of honeys produced in Piedmont from facilities guaranteed about the flower-specificity and quality of honeys has been made. A total of 15 honey specimens were used in this study: 3 Chestnut honeys, 3 Dandelion honey, 3 honeydews, 3 lime tree honeys, 3 wildflower mountain honeys were compared to a medical honey (Medihoney™), produced in New Zealand from manuka tree.
The antibacterial properties of honeys were tested against 2 multiresistant pathogenic organisms: S. aureus methicillin resistant (MRSA) and ESBL E. coli, isolated from wound infections and characterized in our laboratory. In brief all honeys, included Manuka, were screened by agar well diffusion assay (1) and those that showed the highest antibacterial activity, one for each kind of honey, were included in a second step of the study, which involved MIC determination (microdilution assay). Honeys were used undiluted and diluted and an “artificial honey”, consisting of different polysaccharides, was used as control. Finally, we tested the MBC by streak plate method (2).

The initial screening showed that all honeys, except dandelion, had antibacterial activity against S. aureus and less versus E. coli, in agreement with consulted bibliography (2,3).
Among honeys used in the second step, the results obtained by MIC showed a greater activity of honeydew and manuka, when compared to other honeys.

The present study shows that most of honeys produced in Piedmont has high but variable activity against the organisms tested and are more effective against Gram positive than Gram negative organisms. In particular honeydew demonstrates a strong bacteriostatic and bactericidal activity, compareble to Manuka honey.
Since some honeys used in this study showed to possess antibacterial characteristics comparable to those of a honey medicated already commercially, they therefore may be taken into account for the production of medical and surgical devices (dressings, sutures, prostheses).

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AN UNUSUAL CASE OF SPONTANEOUS KIDNEY RUPTURE SECONDARY TO NEMATODE LARVAE DIAGNOSED WITH CONTRAST-ENHANCED ULTRASONOGRAPHY IN A DOG

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To describe the utility of contrast-enhanced ultrasonography (CEUS) for revealing early evidence of an ongoing hemorrhage in a dog with spontaneous kidney rupture and the unusual presence of nematode larvae within the renal parenchyma.

A 9 month old male Boxer dog was examined because of acute onset of hypovolemic shock. There was no known trauma. Venous blood for complete blood count, chemistry tests and coagulation profile was obtained. Subsequently, abdominal ultrasound, analysis of the peritoneal fluid and CEUS were scheduled, followed by laparotomy with nephrectomy of the right kidney. Histological examination and PCR for 18S gene were performed, along with urinalysis and serial fecal tests. After surgery, the dog had been examined regularly up to 1 year.

Physical examination revealed pale mucous membranes, tachycardia with weak peripheral pulse, generalized weakness, abdominal distension and pain on palpation. Laboratory findings were consistent with hemorrhage. Abdominal ultrasonography revealed free fluid and a mass in the cranioventral pole of the right kidney. Fluid removal by abdominocentesis was consistent with free blood. The early arterial phase of CEUS study was characterized by a small area in the renal cortex without enhancement and by multiple septa with strong enhancement extending from the renal cortex into the center of the mass. In the late corticomedullary phase, kidney enhancement was homogeneous without filling defects. Laparotomy confirmed the ruptured kidney. The postoperative course was uncomplicated. A histological diagnosis of severe, interstitial hemorrhage, mesangiosclerosis with endocapillary hypercellularity, and mild multifocal interstitial granulomatous nephritis associated with nematode larvae was posed. PCR resulted negative. No parasite ova in the urine or feces nor clinicopathological abnormalities were detected at follow-up examinations.

The CEUS findings of this dog were compatible with an acute hematoma or hemangiosarcoma (1). An abscess, although possible, was ruled out because of hyperechoic septa which, instead, were suggestive of an active hemorrhage (2).
With regard to nematode larvae, Angiostrongylus vasorum was considered a possibility based on the histological lesions and literature (3). Furthermore, asymptomatic cases, acute bleeding and false negative fecal tests have been recorded (3), as in this case. However, because PCR results were negative, a definitive diagnosis could not be confirmed and remains speculative at best.

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LIMBAL STEM CELLS: A POTENTIAL TOOL IN CORNEAL REGENERATION

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The aim of the study was the identification and characterization of limbal stem cells. In particular, isolation procedure was set up and ability of cells to differentiate in epithelial corneal tissue was investigated. This approach could be a potential therapeutic tool in order to repair corneal injury(1).

Rabbit eyes and retrobulbar fat were sterile collected. The limbal tissue was separated and processed as described by Ghafar 2013. Isolated keratocytes were seeded at the density of 5 x 103 cells/cm2 in specific medium. Plates were incubated at 37°C 5% CO2. Retrobulbar adipose tissue (RAT) was enzymatically digested and cell pellet was seeded and incubated at 37°C 5% CO2. The morphological features of limbal keratocytes were examined daily by using phase contrast microscope. In order to perform a gene expression analysis, the limbal keratocytes monolayers was detached by trypsin-EDTA. Total RNA was extracted and used as template for cDNA synthesis. The expression of Lumican, aldehyde dehydrogenase (ALDH), Collagen type I (Coll I), α-smooth muscle actin (α-SMA) was evaluated using primer sets described in Ghafar 2013 by EVA Green Real-Time PCR amplification. GAPDH was used as housekeeping control gene. Finally, RATSCs were incubated with medium collected from LESC cultures for 7 days. Cells were daily observed in order to evaluate morphological features.

LESCs adhered to surface (24h) and they showed a dendritic, stellate–shaped on day 7. Finally, cells reached confluence on day 25. Retrobulbar adipose tissue stem cells began to adhere 24h after seeding, with a fibroblastic-like morphology. Finally, they reached confluence in 14 days. Cells incubated with LESC medium changed their morphology on day 4, acquiring a dendritic, stellate–shaped. The RT-PCR demonstrated the expression pattern typcal of corneal keratocytes (α-SMA, LCN, ALDH and Coll I), until 4th passage.

Nowadays, several researches and applications have been demonstrated the key role of LESCs during corneal regeneration process either in animals or in human being (3,4,5). This study focused the attention on the in vitro features of two different kind of stem cells: LESCs and RATSCs. In particular, they demonstrated the ability to adapt to in vitro environment and to apparently differentiate in epithelial-like cells. Furthermore, LESCs expressed typical corneal keratocytes markers. These data will be improved by molecular biology analysis, in order to understand the complete expression pattern before and after in vitro differentiation.

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REFERNCE VALUES FOR SELECTED OPHTHALMIC DIAGNOSTIC TESTS AND OPHTHALMIC EXAMINATION FINDINGS OF DONKEY (EQUUS ASINUS RAGUSANUS)

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To establish reference values for the Schirmer tear test 1 (STT 1), the intraocular pressure (IOP) with applanation tonometry (TonoPen-Vet) and to describe clinical aspects of normal donkey eyes. To record the prevalence and document the types of eye disease in population of Equus asinus Ragusanus in Catania, Italy.

Eighty eyes of forty donkey (Equus asinus Ragusanus) of different age and gender were investigated. A full ophthalmic exam including slit lamp biomicroscopy, indirect ophthalmoscopy, measurement of STT 1, measurement of the IOP (TonoPen-Vet) and fundus photographs by Clearview optical imaging system (Eickemeyer) was performed. The normal appearance of the lid, the iris, the lens, the fundus, and the optic nerve disc was evaluated. In the presence of ocular diseases, findings were documented and correlated with factors such as age and gender. Pedigree analysis of donkey affected by cataract was performed.

Twenty eyes (20/80-25%) of donkey foal and sixty eyes (60/80-75%) of adult were examineted. Age range was 2 months-13 years (mean 6); 37 (92,5%) female, 3 (7,5%) male. The median STT 1 was 32 (mean 30,89 mm/min), the median IOP was 18 (mean 18,25 mmHg). The predominant iris color was grey brown in foals, brown in adult. The fundus pigmentation was predominantly green. Potential vision-threatening eye disease wasn't present. The prevalence of ocular abnormalities was 22,5 per cent (18/80 eyes). There were more eye diseases in the left eye compared with the right and older donkeys were more likely to have ocular pathology. Clinically abnormalities included: conjunctival foreign bodies (1/18 eyes-5,55%), stromal fibrosis (4/18 eyes-22,23%), persistent pupillary membranes (5/18 eyes-27,78%), incipient cataracts (7/18 eyes-38,89%) and asteroid hyalosis (1/18 eyes-5,55%). Pedigree analysis showed no evidence of inbreeding as a cause of cataract formation.

The prevalence of ocular diseases was 22,5%, cataract is the most frequent pathology in population of Equus asinus Ragusanus in Catania.

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SLAUGHTERING: THE DIFFICULT TASK OF BALANCING LEGAL ISSUES WITH ETHICAL CONCERNS

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The study aims to analyze the problem of the social acceptability of the practice of slaughter (with particular reference to ritual slaughter), in contemporary Western society, taking into account its prevalent ethical values. It will be offered an examination of: the legal principles applicable to the protection of animals at the time of killing; the legal status of non-human animals (in the light of the different view of them as sentient beings versus mere chattel); the need to reconcile conflicting interests involved, engaging lawmakers in the search for a regulatory response that meets the different instances involved. It will be further discussed some of the terms used by the legislator (such as anxiety, pain, utilities).

It will be analyzed:
- The main soft law documents that contain the guiding principles for the protection of animals kept for farming purposes and for slaughter: 2006-2010 Communication COM (2006) 13, European Conventions ETS no. 102 - 10/05/1979 and STE. 87 - 10/03/1976, Treaty of Lisbon, 2007;
- The regulations for the protection of animals for slaughter: history from the Dir. 74/577/EEC 93/19/CE up to the current Regulation no. 1099/2009/CE.
- Documents that provide economic analysis: SANCO/2049/2008 Commission staff working document Impact Assessment Report
- Ethics reflection papers: National Bioethics Committee (Opinion 19/09/2003), Committee for veterinary bioethical (La macellazione, 2003)
- Lexicographic and scientific definitions of some terms used in Reg. 1099/2009/CE

The European Community guarantees the protection of animals for slaughter and at the time of killing since 1974 and reaffirms the scientific evidence that animals are sentient beings, in accordance with the bioethical reflections. The UE emphasizes also the opportunity to tolerate the traditions of the Member States and local cultural events as such as historical customs and religious rites, excluding them from the scope of the new regulation if the provisions for the protection of animal welfare should alter key parts of them. In the case of ritual slaughter, different needs and subsisting legal and moral obligations must be balanced to guarantee both the right to freedom of religion and animals.
The desire to protect the animals meets so many limitations. In addition there is the difficulty to actually get stronger laws when they contain definitions that are difficult to deal with the complex physiological and behavioral characteristics of the different animals.

The legislation for the protection of animals in killing does not solve all occasions of conflict between the values to be protected.
until attitudes towards animals will also depend on local traditions and rites it will be difficult to adopt more extensive animal welfare rules than those already agreed upon at Community level.

Law past and in force,
National Bioethics Committee (Opinion 19/09/2003), Committee for veterinary bioethical (La macellazione, 2003)

CIRNECO DELL'ETNA: MORPHO-FUNCTIONAL AND PATHOLOGIC OCULAR FEATURES

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The Cirneco dell'Etna is a very old Sicilian canine breed. The aim of this study was to investigate the morpho-functional and pathologic ocular features through a complete eye examination performed on 61 dogs of pure breed, coming from several Sicilian farms.

The average age of animals was 4,16 years (from 2,5 months to 13 years) and they were divided into three groups: 1st group from 0 to 12 months (18%), 2nd from 1 to 6 years (61%) and 3rd more than 6 years (21%). Data were collect in ophthalmic examination sheets.

Results showed that 34 dogs (55,7%) were free from any ocular pathology: most of them presented an amber or light brown iris, some animal had mucoid discharges with normal values of STT, probably related to outdoor life, whereas the non tapetal fundus was poorly pigmented in almost all healthy dogs because of the light color of the coat. The remaining 27 dogs (44,3%) had ocular anomalies divided into non inherited (secondary to exogenous causes, traumatic or supposed infectious) and presumed genetic or inherited diseases. Among the latter, several ocular tissues were affected in different percentages: 13,9% adnexa (KCS, eyelid neoplasm), 51,2%, uvea (uveal cyst, iris atrophy, PPM), 2,3% iridocorneal angle (glaucoma), 20,9% lens (cataract), 7% vitreous (vitreal degeneration, PTVL) and 4,7% fundus (PRA).

Therefore, from data collected, uvea resulted ocular tissue more affected, probably because of characteristic thinness of iris in this breed.

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CIRNECO DELL'ETNA: MORPHO-FUNCTIONAL AND PATHOLOGIC OCULAR FEATURES

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Key words: Cirneco dell'Etna, ocular features, inheritance

The Cirneco dell'Etna is a very old Sicilian canine breed. The aim of this study was to investigate the morpho-functional and pathologic ocular features through a complete eye examination performed on 61 dogs of pure breed, coming from several Sicilian farms. The average age of animals was 4,16 years (from 2,5 months to 13 years) and they were divided into three groups: 1st group from 0 to 12 months (18%), 2nd from 1 to 6 years (61%) and 3rd more than 6 years (21%). Data were collect in ophthalmic examination sheets. Results showed that 34 dogs (55,7%) were free from any ocular pathology: most of them presented an amber or light brown iris, some animal had mucoid discharges with normal values of STT, probably related to outdoor life, whereas the non tapetal fundus was poorly pigmented in almost all healthy dogs because of the light color of the coat. The remaining 27 dogs (44,3%) had ocular anomalies divided into non inherited (secondary to exogenous causes, traumatic or supposed infectious) and *presumed* genetic or inherited diseases. Among the latter, several ocular tissues were affected in different percentages: 13,9% *adnexa* (KCS, eyelid neoplasm), 51,2% *uvea* (uveal cyst, iris atrophy, PPM), 2,3% *iridocorneal angle* (glaucoma), 20,9% *lens* (cataract), 7% *vitreous* (vitreal degeneration, PTVL) and 4,7% *fundus* (PRA). Therefore, from data collected, uvea resulted ocular tissue more affected, probably because of characteristic thinness of iris in this breed.

HYPERLIPIDEMIA IN TWO DONKEYS

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Hyperlipidimia is a metabolic disease caused by abnormal levels of tryglicerids in the blood. Donkeys and ponies are especially sensible to this disease, that can lead to the death of the animals. It can be a primary ailment, or secondary to other illnesses causing anorexia and consequently negative energy imbalance in fat animals.

Two female donkeys were admitted at our hospital during the last twelve months, with anorexia and depression. The blood work at admission showed high levels of tryglicerids only in the first, while the second presented these changes the day before the death, three days after admission. Further diagnostic tests were not performed due to cost restriction, but a tentative diagnosis of secondary hyperlipidemia was made for both donkeys. For the first mare, that had also fever, CBC and blood smear evaluation showed signs of pancytopenia and blood cell neoplasm, confirmed later on during the necropsy. The second, an unusually fat animal, showed signs of liver failure, with elevated levels of all liver enzymes, and hyperglycemia. Necropsy was performed on both animals: a diagnosis of myeloma was made for the one with fever with abnormal cells in bone marrow, liver and spleen, while in the second a tentative diagnosis of equine metabolic syndrome (EMS) was made, because of clinical signs and lipid infiltration in various organs.

Hyperlipidemia is a serious condition of equids, caused by a negative energy imbalance, consequence of anorexia in fat animals. It could be a primary condition, due to lipomobilization, or it could be secondary to systemic diseases. Myeloma is a lethal illness, quite rare, causing proliferation of abnormal cells derived from myeloid hematopoietic cells; it is a condition typical of younger animals, showing aspecific signs, with a poor prognosis, that often leads to either death or euthanasia. Equine metabolic syndrome, on the other hand, is typical of pony breeds, especially fat animals, unfortunately in our donkey it was not possible to make a definitive diagnosis, but the lipid infiltration of the organs, the history and the cresty neck make EMS a likely cause of the disease. Treatment of the hyperlipidemia is based on management of the primary condition and feeding the animal as soon as possible. Prognosis is related to the time of the diagnosis and to the underlying illness, and is usually guarded for the life of the animal.

Hyperlipidemia should always be contemplated in obese ponies and donkeys and treated as soon as possible, to avoid complications that could lead to the death of the animal.

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EVALUATION OF CONGIUNCTIVAL FLORA IN DONKEYS FROM SICILY

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The use of ass's milk has been recently revalued as an alternative food for infants with cow's milk protein allergy (CMPA), the most common food allergy in childhood. Clinical data suggest that infants with CMPA could tolerate donkey's milk up to an age at which bovine milk could be reintroduced without complications. However, such milk requires appropriate nutritional modification before administration to infants, and its safety profile must be carefully evaluated. The "Ragusano" breed was officially recognized in 1953, after a long selection carried out by the Sicilian Institute of Horse Breeding. According to the data from the Safeguard for Agricultural Varieties in Europe (SAVE) Monitoring Institute in 2002, the breed is one of the 11 donkey breeds at high-risk of extinction. The aim of this study was to evaluate the health status of donkeys from Sicily by the examination of the bacterial flora present in the normal conjunctiva.

A total of twenty-seven healthy donkeys of "Ragusano" breed housed in different locations of Sicily were sampled during summer 2013. Donkeys ranged from 2 to 13 years of age. Conjunctival swabs were obtained from both eyes of each animal and were brought to Zooprophilactic Institute of Sicily. Bacteriological exams were carried out according to standard procedures. Antibiotic susceptibility testing were carried out using Kirby-Bauer method.

Only three specimens showed the presence of few colonies of Moraxella spp., Bacillus licheniformis and Pseudomonas spp.. These last two are environmental bacteria widespread in nature. Only Moraxella could have a pathogenic potential even if the donkey did not show any pathological lesion. Antibiotic diffusion test was carried out for every isolated strain and the results showed that all the strains were sensitive to every used antibiotic (at least seven for every strain).

Donkeys can become carriers of resistant determinants, with the risk of
a possible dissemination and transmission of pathogens. Humans can acquire saprophytic and pathogenic flora by direct contact (for farmers) or by consumption of donkey milk. So, the abiding evaluation of the health status of donkeys is particularly important for the safeguard of human health.

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TRICUSPID ANNULAR PLANE SYSTOLIC EXCURSION (TAPSE) EVALUATION IN DOGS WITH CHRONIC DEGENERATIVE VALVULAR DISEASE WITH AND WITHOUT PULMONARY HYPERTENSION

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The aim of the study was to investigate the TAPSE in dogs with chronic degenerative valvular disease (CDVD) at different stages of heart failure (HF) and pulmonary hypertension (PH).

Dogs were prospectively enrolled and underwent a complete cardiologic evaluation, including physical examination, thoracic radiography, EKG, and trans-thoracic echocardiographic examination (TTE). The TTE included 2D and M-mode evaluation of the left ventricle, Doppler interrogation of trans-valvular blood flows and Tissue Doppler Interrogation (TDI) of the lateral and septal mitral annulus and the tricuspid annulus.
Healthy (control) dogs and dogs with CDVD with combined mitral and tricuspid regurgitation (TR) but without other cardiovascular disorders were selected. Evidence of PH was based on TR jet Peak velocity (TRVMax) >2.8 m/s. In dogs with CDVD, HF severity was classified according to proposed guidelines (Atkins C., 2009). Dogs with CDVD were also subdivided in: No PH, if TRVMax ≤2.8 m/s; Mild PH, if TRVmax >2.8 m/s and ≤3.5 m/s; Moderate PH, if TRVMax >3.5 m/s and ≤4.5 m/s; and Severe PH, if TRVMax >4.5 m/s.
To obtain TAPSE, a 4-chamber apical view, optimized for right ventricular inflow was used, and an M-mode cursor was placed through the lateral tricuspid annulus. TAPSE was measured on M-mode images as the total displacement of the tricuspid annulus from the deepest point in diastole to the highest point on systole. The parameter was measured three times and the average value was used for statistical analysis.
Correlations between TAPSE and body weight (BW), body surface area (BSA), heart rate and other echocardiographic parameters were investigated using Pearson coefficient and those normally distributed, were analyzed using an analysis of covariance (ANCOVA) to assess the differences among PH groups and HF groups, corrected for BSA. P values < 0.05 were considered significant.

103 dogs were recruited: 8 were free from heart disease, 56 had CDVD but No-PH, 25 had CDVD and Mild-PH, 11 had CDVD and Moderate-PH and 3 CDVD and had Severe-PH. According to the ACVIM classification of HF, 8 dogs were in class A, 53 in class B1, 22 in class B2, 17 in class C and 3 in class D. Average BW was 14.5 Kg (Range 2.6 – 72 kg); the average age was 9.5 years (range 0.7- 16.1 years).
TAPSE values were significantly correlated with BW and BSA, (Pearson correlation coefficient 0.49 and 0.52, respectively). TAPSE values were also correlated with some echocardiographic indices of left and right ventricular systolic and diastolic function. ANCOVA analysis did not evidence any significant difference in TAPSE values among dogs of different PH and HF groups.

Although TAPSE is significantly correlated with some right ventricular parameters it does not seem to play an important role in dogs with CDVD and PH

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SERUM THIAMINE IN DOGS WITH MITRAL VALVE DISEASE

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Mitral regurgitation (MR) caused by myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs. In human medicine MMVD is associated with clinically significant thiamine deficiency. The aim of the present study was to assess thiamin concentration in whole blood samples of dogs with mitral valve disease through HPLC method and to evaluate its correlation with echocardiographic findings and severity of heart failure.

The present study was carried on at the Department of Veterinary Science of Pisa University and at Veterinary Medical Teaching Hospital of Chungnam National University between July 2012 and April 2013. 100 dogs (42 females and 58 males) were enrolled in this study. The control group consisted of 30 healthy dogs (20 Beagle, 5 mixed breeds, 2 Miniature poodles, 2 Shih-tzu and 1 Miniature pinscher). The MMVD group included mixed breed (n=25), Beagle (n=6), Chavalier King Charles Spaniel (n=5), Miniature pinscher (n=4), Shih-tzu (n=4), English setter (n=4), Maltese (n=4), Yorkshire Terrier (n=3), Espaniel Cocker (n=4), Poodle (n=4), Espaniel Breton (n=3), Chihuahua (n=2), Pomeranian (n=1) and Spitz (n=1). Dogs included in this study had to be > 3 years old and between 2-26 kg of body weight. Each dog was submitted to complete clinical and cardiological examination, including complete echocardiographic exam. Dogs affected by mitral valve disease (MVD)) were staged according to ACVIM Consensus Statement, JVIM, 2009 in order to be divided into group B, C and D. Blood samples were collected and whole blood stored at -80°C till HPLC analysis.
Data were statistically analyzed through MedCalc® version 12.5.0.0.

Mean serum thiamine in clinically healthy dogs was 96.53+/- 22,92, in group B 77.61 +/- 17.68, in group C 86.43+/-31.47 and in group D 90.38+/-33.42. No statistically significant difference in serum thiamine concentration was found among clinically healthy dogs and MVD dogs at different stages of the disease, with the exception of group B ($p<0,01$).

In the present study, dogs affected by MVD seemed not to develop severe thiamine deficiency. Although in this cohort of dogs a significant reduction in serum thiamine was not reported, B vitamin supplementation resulted in a statistically significant improvement of cardiac performance.

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ASSESSMENT OF THE COMPLETE BLOOD COUNT IN THE CHRONIC KIDNEY DISEASE (CKD) OF DOGS AND CATS

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Dogs and cats with CKD typically have normocytic-chromic non-regenerative anemia and the severity is proportional to the kidney function loss(1,2). Less information is known about leukogram and thrombogram modifications(3). The complete blood count (CBC) in relation to CKD stages according to International Renal Interest Society (IRIS) has been evaluated.

445 dogs and 254 cats (different breed, age and sex) affected by CKD staged according to IRIS were included. CBCs results and Serum Total Protein (STP) levels were retrospectively investigated at the initial enrollment. Data were analyzed by 1 way ANOVA, Dunn's test (Dt) or Tukey's test (Tt), and Chi square (X2).

Dogs studied were(%): most represented breeds Mixed (31), Boxer (7) and German Shepherd (6); Males (58), Females (42); ages, 8-13 yo (54.5); IRIS stage 1 (1, omitted), 2 (46), 3 (36), 4 (17).
In relation to IRIS stage worsening the following parameters were significant (ANOVA, $p<0.05$): decrease of RBC, Hct and Hgb (Dt for all IRIS, $p<0.01$); decrease of reticulocytosis (Dt, IRIS 2-4, 3-4, $p<0.01$); increase of Neutrophil, decrease of Lymphocyte, Eosinophil and Platelet counts (Dt IRIS 2-4 & 3-4, $p<0.05$-0.01). Comparison of Non-Anemic vs. Anemic dogs showed increase of normocytic-chromic anemia (X2, $p<0.01$).
Cats studied were(%): most represented breeds D/SH/LH (76), Persian (9), and Siamese (8); Males (50), Females (50); ages peaks at 14, 4, 7 yo (34); IRIS stage 1 (3, omitted), 2 (72), 3 (11), 4 (14).
In relation to IRIS stage worsening the following parameters were significant (ANOVA $p<0.05$): decrease of RBC (Dt, IRIS 2-3 & 2-4 $p<0.01$), Hct and Hgb (Tt, IRIS 2-3 & 2-4 $p<0.01$); increase of total WBC, Neutrophil (Dt, IRIS 2-3 & 2-4, $p<0.01$) and Monocyte (Dt, IRIS 2-4, $p<0.05$), decrease of Eosinophil counts (Dt, IRIS 2-4 & 3-4, $p<0.01$); increase of SPT level (Dt, IRIS 2-4 & 3-4, $P<0.05$). Comparison of Non-Anemic vs. Anemic cats showed increase of normocytic-chromic anemia (X2, $p<0.01$); PLT aggregates decreased (X2, $p<0.05$).

Erythroid section involvement in dogs and cats related to the worsening of IRIS stage was confirmed, as already reported(1,2). A progressive increase of STP levels in relation to IRIS worsening in cats, probably due to dehydration progression, was observed. The PLT aggregates in cats tends to be reduced in relation to IRIS, probably due to PLT function loss. In both species, the median eosinophil count in reference ranges despite its reduction related to IRIS worsening was evidenced. Similar results were reported in human studies(4). In canine and human investigations a progressive lymphopenia related to CKD severity was reported(3,4).

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STERNAL ASPIRATION OF BONE MARROW IN DOGS: A PRACTICAL APPROACH FOR CANINE LEISHMANIASIS DIAGNOSIS AND MONITORING

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The aim of the study is to describe a technique for sternal bone marrow aspiration safe and easy to perform and to provide results indicating the usefulness for parasitological examinations necessary to CanL diagnosis and monitoring.

Dog is positioned in right lateral recumbency. Third or fourth sternebra is identified by palpation, clipped, and aseptically prepared.No pharmacological sedation is usually required. The needle is threaded through the skin up to the stenerbra's wall and firmly pushed forward, while rotating. Approximately 5 ml of vacuum is applied for 5-10" depending on the volume of bone marrow required, usually around 0.5 ml. The approached area is disinfected.

We reviewed data collected during a 8 year period on 2,500 bone marrow sternal aspirations performed in 889 dogs for leishmaniasis diagnosis and monitoring. Dogs aged 6 months-14 years were of both sex and of different breeds.
1716 samples were obtained from 387 dogs enrolled in five clinical trials. The studies consisted of long-term follow up evaluation starting from a naïve condition followed by natural exposure to infection. 371/1716 bone marrow n-PCR were positive (21.6%). N-PCR was constantly positive when infection was confirmed by IFAT >1:160, with 100% concordance between the 2 methods (1).
The remaining 502 dogs (748 bone marrow samples) were pets admitted to bone marrow aspiration after owner's consent for parasitological investigations. Amastigotes were detected in 344/748 smears (46.0%),such rate being higher in dogs with advanced stages of CanL and/or high IFAT titres. Also in these dogs concordance between IFAT >1:160 and cytology was 100%.

Several veterinarians hesitate to approach bone marrow aspiration as a routine due to lack of practice and fear for side effects. Bone marrow collection could increase sensitivity of direct diagnostic methods; demonstrate parasite spread; be useful for CanL staging, prognosis and and for drug failure; evaluate hematologic disorders (2,3). Sites available for bone marrow sampling include dorsal iliac crest, femoral/humeral shaft, costochondral junction. The sternal site it is not listed among the preferred sites or it is considered dangerous. We never encountered problems despite our large canine population. The described technique can be considered safe and easy to perform, providing a good representative sample for parasitological diagnosis of CanL and evaluation of hematopoiesis of the patient.

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ULTRASOUND ABNORMALITIES IN 25 DOGS SUBMITTED TO INTERMITTENT HEMODIALYSIS

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The aim of the present study was to evaluate the prevalence of ultrasound abnormalities in a group of dogs submitted to intermittent hemodialysis. Secondary objectives were to evaluate the association among ultrasound abnormalities and different stages of the disease, and ultrasound abnormalities and mortality rate.

25 dogs of different breed, gender, age and weight presented to the Centro di Emodialisi e Purificazione Ematica Veterinaria (CEPEV) for acute kidney injury (AKI) were submitted to intermittent hemodialysis. All patients required renal replacement therapy (RRT) and were classified according to AKI staging1,2 on the basis of plasma creatinine. Abdominal ultrasound was performed in all subjects before starting hemodialysis. Data were analyzed through GraphPad Prism®.

6/25 dogs (24%) developed AKI secondary to ethylene glycol intoxication, 2/25 (8%) to grape intoxication, 2/25 (8%) to Leptospirosis infection, 2/25 (8%) to pyelonephritis. In 13/25 dogs (52%) the cause of AKI was unknown. Stage AKI 1 enrolled 1/25 dogs, AKI 2 2/25 dogs, AKI 3 2/25 dogs, AKI 4 13/25 dogs and AKI 5 7/25 dogs. Overall survival rate was 56% and mortality rate 44%. According to AKI staging, survival rate was 100% in AKI 1 and 3, 50% in AKI 2, 54% in AKI 4 and 43% in AKI 5. Although no statistical association was found, the prevalence of ultrasound abnormalities seemed to increase with the progression of AKI. No ultrasound abnormalities were found in AKI 1, while in AKI 2 and 3 the most frequent abnormalities were represented respectively by cortical-medullary hyperechogenicity and cortical hyperechogenicity. In AKI 4 the most frequent abnormality was pyelectasis (54%), in AKI 5 cortical-medullary hyperechogenicity (58%). No significant difference was found between survived and not survived patients, with the exception of nephrocalcinosis, which showed a significantly higher prevalence (p=0.02) in not survived dogs.

In the present cohort of dogs, pylectasis and cortical-medullary hyperechogenicity resulted the most frequent abnormalities. Although no correlation was found with the progression of the disease, the prevalence of different ultrasound abnormalities increased in stages AKI 4 and 5 compared to early stages. Nephrocalcinosis seemed to be the only ultrasound finding significantly related to poor prognosis and it was found in 37% of not survived dogs, while it was not present in survived dogs. Although ultrasound represents a powerful tool in the diagnosis of both AKI2 and chronic kidney disease (CKD), the absence of ultrasound abnormalities in patients requiring RRT should not discourage to treat them, especially if ethylene glycol intoxication is suspected.

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DIAGNOSIS AND MANAGEMENT OF EQUINE UROLITHIASIS

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Urolithiasis is quite rare in horses, and it could be an incidental finding in slaughtered animals. In this report, we will describe diagnostic procedure and therapy of urolithiasis.

Between 2011 and 2013, 4 horses, different for breed and age, were admitted at our hospital with a diagnosis of urolithiasis, 3 geldings with bladder calculosis, and 1 female with ureteral stones. Clinical signs were hematuria and stranguria in the males, while in the mare, it was an incidental finding after a colic surgery for intestinal problems. Blood work was normal at admission for all horses, but one of the males developed renal failure, during the hospitalization. Diagnosis was based on ultrasonografic and endoscopic examination of the urinary tract. The bladder calculi were removed using laparoscopy, while the ureteral one passed in the bladder after therapy with NSAIDs and antibiotic, and was then taken out with endoscopic guidance. The outcome was good for the female and one of the males, while a second gelding was euthanized due to complication during the recovery after surgery, while the last one died because of renal failure, developed after admission.

Urolithiasis is a disease of the horse that present itself with different clinical signs, the most common being hematuria and stranguria. Bladder calculosis is more common in the male, due to the size of the urethra. Ultrasonographic and endoscopic examination are the gold standard for the diagnosis, because they allow to visualize the stone and check the mucosa of bladder and urethra for other causes of hematuria. Important is also the blood work, to evaluate the consequences the stones could have had on the renal function, and to give a prognosis and guide in choosing the best therapy. Laparoscopy has proven to be an efficient method to remove the calculus, with minimal consequences for the horse. The prognosis is quite good, provided that the condition is treated as soon as possible, to avoid the development of renal failure.

Ultrasonography and endoscopy are very useful means of making a diagnosis of urolithiasis. Laparoscopy has proven to be an effective way to treat the condition, provided it is performed as soon as possible, to avoid development of renal failure, that could lead to the death of the animal.

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THROMBOSIS IN 46 DOGS: RETROSPECRIVE REVIEW AND CLINICAL COMMENT

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Thrombotic complications of naturally occurring disease processes are being recognized with increasing frequency in canine medicine. Through retrospective studies of dogs with confirmed thrombosis, the most common underlying diseases predisposing to thrombus formation can be identified. These include immune-mediated diseases, protein-losing nephropathy and enteropathy, various type of neoplasia, sepsis, and cardiac disease. Thrombi localized to the pulmonary vasculature, the splenic vein, the distal aorta, portal vein, and the cranial vena cava have been described retrospectively1. The aim of the study is identify concurrent diseases and conditions in dogs with thrombosis during abdominal ultrasound

Medical records from August 2009 to April 2013 were retrospectively reviewed for 46 dogs with arterial or vein thrombosis identified by abdominal ultrasound examination. Data were reviewed for signalment, medical history, clinicopathologic testing, diagnostic imaging, and clinical diagnosis.

The most common site of thrombosis was splenic vein (45%). The most common concurrent disorder was neoplasia (56%). Haemolymphatic (26%) and gastrointestinal (24%) diseases were the most common diseases. Most of dogs with gastrointestinal diseases had suspected inflammatory pathogenesis (64%). Multiple site thrombosis were observed in a few of dogs (15%), most of them affected by hypercortisolemia (43%).

Arterial and/or vein thrombosis identified by abdominal ultrasound examination may be correlated with one or more pathologic condition. Inflammatory gastrointestinal diseases may be predisposing to thrombus formation. Hypercortisolemia may lead to multiple site thrombi formation. Studies evaluating anticoagulant therapy in the management of incidental thrombosis are warranted. It is advised to perform routine abdominal ultrasound investigation for vasculature impairment in all dogs with the predisposing disorders as above described.

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ATRIAL FIBRILLATION IN THE HORSE: REVIEW OF 27 CASES

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Atrial fibrillation (AF) is a common arrhythmia in horses and it is the most common cardiovascular cause of poor performance in the equine athlete. AF seems to be related with a “re-entry” mechanism with or without underlying cardiac diseases and there do not appear to be any gender predisposition. Aim of the present report is to review the epidemiological, clinical and therapeutic aspects of AF in 27 horses.

The horses had an history of poor-performance (6 horses), cardiovascular disorders (19 horses), exercise induced pulmonary hemorrhage (1 horse) and collapse (1 horse). The symptoms were present for less than 1 month in 17 cases and for longer than 1 month in 10 horses. The horses were 16 trotters and 11 warmblood; 10 males, 10 females and 7 geldings with a mean body weight of 497,11±61,3 kg and a mean age of 8±6 years. All horses underwent a thorough physical examination, laboratory evaluation, electrocardiography (ECG), 24 h Holter recording (HR) and echocardiography (ECC). According with the results obtained 17 horses were treated for AF (AFt), 9 horses were not treated and spontaneous resolution of AF was recorded in 1 horse. 15 horses received 22 mg/kg quinidine sulfate (QS) by nasogastric tube every 2 hours until cardioversion (CV); 1 horse, since QS was ineffective, received amiodarone cloridrate (Am) intravenously (De Clercq et al 2006) until CV, and 1 further AFt case received Am first and then QS. During treatments the horses were monitored by ECG.

All horses had normal body temperature (37,8±0,3 °C); on pulse palpation 12 had reduced intensity and amplitude and all had irregular rhythm. In all cases, heart auscultation revealed an irregularly irregular rhythm and murmurs were detected in 10 horses. ECG and HR confirmed the arrhythmia with mean rate of 44±11 bpm. ECC showed increased ventricular and/or atrial diameters in 4 horses, reduced left ventricular fractional shortening in 9 horses, valvular incompetence in 19 horses. In the 15 QS treated horses CV was achieved after different number of treatments: 2 in 1 case, 3 in 4 cases, 4 in 2 cases, 5 in 1 case and 6 in 6 cases. 2 out of this 15 horses received a double QS treatment at 1 week interval and in these CV was obtained after a total of 6+5 and 6+6 treatments. In the QS+Am case CV was obtained after 26 hours of Am administration. In the Am+QS case Am was discontinued due to signs of toxicity and a subsequent QS administration was ineffective. The treatment was effective in 16 out of 17 horses.

In our review QS showed its efficacy for AF in 94% of cases without side effects. The Am+QS treated horse did not convert to sinus rhythm probably due to chronic AF. Probably, the high rate of success obtained can be related with the accurate ECC selection of the cases. However, since QS is becoming difficult to obtain, other cardioverting techniques or drugs like Am, which is currently used in horses for chronic cases of AF, need to be further investigated in the equine patient.-

NOSTRIL MASS CAUSED BY LEISHMANIA INFANTUM IN A DOG

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The aim of the study is to describe a clinical case of a dog infected by Leishmania infantum with a diffuse nostril mucosal involvement, treated successfully with miltefosine and allopurinol.

An English Setter female, 7 years old, was referred to the Hospital of our Faculty for the evidence two months before of unilateral mucous nasal discharge, followed by a mass appearance. No response to antibiotic treatment was obtained. History revealed a previous anti-Leishmania IFAT titre at borderline cut-off (1:160), with no specific clinical signs. Apart for the presence of ulceration at nasal muco-cutaneous margin and nostril mucosal hypertrophy, clinical examination showed no relevant signs. Main differential diagnosis included nasal neoplasia and rhinitis. Routinary haematological, biochemical and urinary examination were performed, together with anti-Leishmania IFAT , bone marrow and limph-node aspirates. Nasal mass was examined by brushing cytology and histological examination after biopsy. Specific culture for Leishmania was also performed by nasal tissue

Laboratory parameters showed several clinicopathological alterations: mild anemia, increase of ALT and total protein values, inversion of albumin/globulin ratio, proteinuria and increase of UPC ratio. Parasitological exams proved a positivity to anti-Leishmania IFAT (1:640), lymph-node and bone marrow aspirates was negative. Brushing cytology revealed the moderate presence of inflammatory cells; no neoplastic cells and amastigotes were detected. Histological examination showed a proliferation of macrophages, lymphocytes, plasma cells and very rare amastigotes. The biopsy culture was positive for Leishmania infantum promastigotes. Dog was treated by miltefosine and allopurinol. After the first month a clear improvement of general condition associated to a reduction of nostril mass was detected; after 2 months a complete disappearance of the mass was obtained.

Canine leishmaniosis is a systemic disease, characterized by viscerocutaneous signs. Mucosal involvement is considered rare, both in dog and in human. There are several hypothesis that could explain this possible localization, the most accepted are the immunodepression of the host and/or the particular tropism of some strains but there is no definitive confirmation. The particularity of our case is due to: 1) the absence of systemic disease and the presence of mucosal involvement in a dog in good health condition; 2) the response to conventional therapy. Veterinarians should always include canine leishmaniosis in the differential diagnosis of mucosal and tumor-like lesions.

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ABDOMINAL ULTRASONOGRAPHIC FINDINGS ASSOCIATED WITH CANINE LEISHMANIOSIS: A RETROSPECTIVE REVIEW OF 31 CASES.

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Canine leishmaniasis (CanL) by Leishmania infantum is a polysystemic disease widely distributed in many Mediterranean countries which can be fatal if left untreated. In endemic areas a high percentage of asymptomatic/pauci-symptomatic dogs have been registered1,2. In human patients with visceral leishmaniasis the state of parenchymatous organs have been investigated using ultrasounds in one study3 and interesting focal macronodular lesions in the liver and/or spleen have been described in single cases4,5,6,7. Differently no data are actually available on abdominal ultrasonographic findings registered in CanL except for a single case8. The aim of the present study is to characterize the state of abdominal parenchymatous organs of dogs affected by leishmaniasis using ultrasonography.

Thirty-one dogs naturally infected by L. infantum were retrospectively enrolled in the study. Inclusion criteria were the manifestation of at least one clinical-pathological sign referred to CanL associated to the presence of amastigotes in the lymph-node smears, no evidence of other diseses and the presence of an ultrasound examination at diagnosis and eventually in follow up. Morphologic changes of abdominal organs, including size, focal or diffused lesions and change in echogenicity and echo-texture were recorded.

The most common pathological findings were: spleen from mild to severely enlarged registered in 15 dogs appearing hypoecoic or with a coarse hypoechoic parenchimal pattern; hyperechogenicity of renal cortex revealed in 13 dogs and hepatomegaly in 5. Interesting findings were: enlarged abdominal lymph-nodes described in two dogs, a honeycomb splenic parenchimal pattern described in two dogs and a small liver with irregular margins suggestive of a chronic process revealed in other two dogs. The echographic follow-up post treatment was available only in 9 dogs showing a reverse to normal of ultrasound pathologic findings (i.e: honeycomb aspect of the spleen disappeared; enlarged organs reverse to normal size).

Pathological findings are discussed. Results of this study suggest that ultrasonography could represent a further means in the diagnosis and monitoring of dogs affected by leishmaniosis; in particular it could contribute to increase the suspicion of infection in pauci-symptomatic dogs and be useful to monitor the efficacy ot treatment. Focal macronodular lesions in spleen and liver (as described in humans) were not registered but the study is ongoing to collect data on an enlarged population.

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SEARCHING FOR SHORT EPITOPIC SEQUENCES IN CANINE PEMPHIGUS FOLIACEUS: PRELIMINARY RESULTS

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The present study aims to identify short peptide sequences characterized by a low level of similarity to the canine proteome, and responsible for autoimmune response that characterizes canine pemphigus foliaceous (cPF). As already demonstrated by several authors, in the human model of pemphigus foliaceous and pemphigus vulgaris short peptide sequences from the two antigens of PF and PV (Dsg1 and Dsg3 respectively) with low similarity to the host proteome (mouse and human), are endowed with a high epitopic power (Kanduc, 2009; Kanduc, 2008; Lucchese et al., 2006; Angelini et al.,2006).

Five sera from dogs affected by PF were tested with Dot-blot Immunoassay to evaluate the presence of autoantibodies against two peptides with low similarity to the dog proteome (Canfa, Canis Familiaris): Dsg1_CANFA49-60 and Dsg3_CANFA48-59. Two peptides with high similarity to the dog proteome were employed as negative controls : Dsg3_CANFA189-203 and Dsg3_CANFA372-379. The same peptide platform was used to test sera from healthy dogs.

All the dogs with PF showed antibody reactivity to the low similarity peptide Dsg3_CANFA48-59. No positivity was detected against the low similarity peptide Dsg1_CANFA49-60 and the two high-similarity peptides Dsg3_CANFA189-203 and Dsg3_CANFA372-379. No sera belonging to healthy dogs has recognized the two peptide sequences with low similarity neither the two high-similarity peptides.

The data presented in this study should be interpreted as preliminary results of a larger research project which is still in progress. In fact, thanks to the collaboration of many dermatologists throughout the country we are expanding our study to obtain a statistically significant number of samples. However, to date, we report the evidence that circulating antibodies present in the sera of dogs with PF recognize the Dsg3_CANFA48-59 low similarity peptide sequence. Further studies need to confirm our data and give us a better understanding of the molecular mechanisms at the basis of the PF.

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IDENTIFICATION OF STAGE V IN CANINE LYMPHOMA: COMPARISON OF THREE METHODS

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Lymphoma is the most frequent hemopoietic neoplasm of dogs. B-lineage high grade lymphomas represent the majority of cases (1,2) and DLBCL is the most frequent form (3). Clinical staging is a key point in the diagnostic process determining the degree of extension of the tumor and playing an important role for prognosis and therapy (4). The evaluation of bone marrow and peripheral blood infiltration is mandatory for a complete staging allowing the identification of stage V disease according to the WHO system (5).
Aim of the study was to compare three different methods for detecting peripheral blood infiltration in patients with DLBCL

100 cases of DLBCL were included. Peripheral blood was submitted to the following analyses: CBC with ADVIA 120 (Siemens), MGG-stained smear evaluation, flow cytometric immunophenotyping. Percentages of LUC and BASO (ADVIA 120), blasts out of a 200 cell differential count (MGG-stained smears), CD21+ large cells (flow cytometric immunophenotyping) were recorded. Differential counting on MGG-smears were performed both by an experienced and an unskilled haematologist; atypical lymphocytes and quantity of blasts at the thin edge of the smear were also considered, respectively. For each method specific cut-off values to define stage V were calculated on 15 healthy dogs. Method comparison analyses (Passing Bablock regression and Bland-Altman plots) were carried out and accuracy in defining stage V disease was calculated for each method. Flow cytometry was used as reference method in both cases.

All the methods except blast count by unskilled hematologist reported significant constant and/or proportional error in quantifying neoplastic circulating cells. All methods presented very wide limits of agreement.
All methods reported a low concordance with flow cytometry in detecting stage V. LUC and BASO presented 100% specificity but a very low sensitivity. The differential count by the unskilled haematologist reported the best sensitivity. Overall evaluation, considering likelihood ratios and diagnostic odd, indicated the differential count by the skilled operator as the best method; ROC curve reported 1% as the best cut-off value with sensitivity and specificity of 63-68% and 93-96%, respectively.

None of the methods considered resulted to be an adequate substitute for flow cytometry in quantifying blood infiltration. Thus, flow cytometry remains the first choice to define stage V in lymphoma patients. LUC and/or BASO percentages from ADVIA 120 are reliable in case of positive result. A blast number >1% in a 200-cell differential count has moderate sensitivity and high specificity. Anyway, the methods evaluated cannot be used interchangeably for the quantification of blood infiltration

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EFFECTS OF SYSTEMIC DRY PRIMIPAROUS MEDITERRANEAN BUFFALO (BUBALUS BUBALIS) TREATMENT WITH PENETHAMATE HYDRIODIDE ON UDDER HEALTH AND MILK YIELDS

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The effects of penethamate hydriodide (Mamyzin®, Boehringer Ingelheim, Ingelheim, Germany) on non-lactating primiparous Mediterranean Buffaloes (Bubalus bubalis) were evaluated in the same farm in south Italy.

An intramuscular administration of 10 million U.I. was performed in 20 buffaloes 7 day pre-partum (treated group - TG), while others 20 animals, left untreated, were enrolled as control group (CG). The animals were followed during all the milking and evening milk samplings was performed at days 10, 30 and 60. Somatic cell count values (SCC) were evaluated on composite milk samples (4-quarters pool), while bacteriological culture (BC) and California Mastitis Test (CMT) were performed on quarter milk. Electrical conductivity (EC) and milk daily productions were always recorded after milking. According to several studies (Moroni et al. 2006), quarters producing milk with SCC values > 200'000 cells/ml, with a positive bacteriological culture, were considered as affected by mastitis and in end point phase. After 60 days post partum, composite milk samples from each primiparous were collected for a monthly SCC and BC until the milking end.

During the whole study 360 SCC, 720 BC and CMT, 360 milk daily production values were recorded. At day 10 postcalving, no mastitis was developed by 90% of animals (18/20) and 97,5% of quarters (78/80) in the treated group, versus 70% (14/20) and 85% (68/80) in the control group respectively. At day 30 postcalving, no mastitis was developed by 94,5% of animals (17/18) and 98,7% of quarters (71/72) in the TG, versus 78,6% (11/14) and 91,4% (51/56) in the CG, respectively. At day 60 postcalving, no mastitis was developed by 94,1% of animals (16/17) and 98,5% of quarters (67/68) in the TG, versus 91% (10/11) and 95,4% (42/44) in the CG, respectively.
At the end of two months considered, the incidence of animals and quarters did not develop mastitis was the 80% (16/20) and the 83,7% (67/80) in the TG, respectively, while only the 50% of animals (10/20) and the 47,5% (38/80) of quarts, in the TC ($p<0.01$). A significant decrease in SCC was observed in TG vs. CG ($p<0.05$) at day 30 postcalving. No significant differences in SCC values and milk productions were detected between 90 days and the milking end.

Buffalo's mastitis is one of the most costly diseases in the dairy industry causing deleterious effects on Mozzarella cheese productions (Moroni et al. 2006). In our experimental condition, the Mamyzin® administration before calving shows a good effectiveness against the most common udder-specific pathogens causing mastitis in primiparous Mediterranean Buffaloes. A higher efficacy was observed post 10 and 30 days of milking than 60 days. Further, the decrease in SCC observed proves as the antibiotic could improve the quality of buffalo milk.

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DIAGNOSIS OF CANINE GASTRIC ADENOCARCINOMA BY SQUASH PREPARATION CYTOLOGY

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Adenocarcinoma (ADK) is the most frequent gastric tumour in the dog (1). Clinical signs and laboratory results are unspecific (2); endoscopy and histopathologic examination of gastric biopsies are required to reach a diagnosis (3-5). A reliable cytologic examination would shorten the time to diagnosis, thus resulting particularly helpful since gastric ADK is often suspected when patient conditions are yet compromised. Few publications exist on cytological evaluation of gastric diseases (6-12) and no reports are present using squash technique.
Aim of the study is to describe cytological features of canine gastric adenocarcinoma and to evaluate the performance of a new approach using squash preparations

Squash preparations of gastric biopsies from 100 dogs were reviewed in order to detect the presence or absence of specific features (signet ring cells, cytoplasmic microvacuolation, neutrophilic infiltration, lymphocytic infiltration, necrosis, cell pleomorphism, atypical mitoses, tubular or acinar cell arrangement and single cell distribution of epithelial cells. Basing on association with ADK, concordance between cytologists and specificity and sensitivity in detecting ADK, two features were selected and the performance of their assessment as parallel tests in diagnosing ADK was evaluated. Histopathologic diagnosis was used as reference result.

Features associated only with ADK were the presence of signet ring cells, cytoplasmic microvacuolation, cell pleomorphism and single cell distribution of epithelial cells. The parallel assessment of the presence of signet ring cells and microvacuolation reported the following performances in recognizing ADK: accuracy=0.939; k=0.863; Se=0.938; Sp=0.939; PPV=0.882; NPV=0.969; LR+=15.47; LR-=0.07; diagnostic odds ratio=232.5

Squash preparation of gastric biopsies is a good technique in gastric cytology. Signet ring cells and cytoplasmic vacuolation are characteristic of canine gastric ADK and their parallel evaluation allows a rapid and reliable diagnosis of the tumour

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NEONATAL AND MATERNAL EVALUATION OF OH CONCENTRATION AND BIOLOGICAL ANTIOXIDANT POTENTIAL IN THE EQUINE FETO-MATERNAL UNIT

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To evaluate the protective role of placenta against fetal oxidative stress in the horse.

68 mares and foals were included in the present. After delivery, a maternal, umbilical and foal venous blood sample was drawn and collected into heparinized tubes. d-ROMs and (BAP) was evaluated. Statistical analysis: OH and BAP concentations were expressed as X±SD. ANOVA and LSD multiple test were performed to evaluate differences inter-groups . Pearson test was also performed to evaluate correlation between d-ROMs and BAP. Significancy was set at p<0,05.

OH and BAP concentrations in mares, umbilical cord and foals are reported in table 1. Statistical analysis showed significant differences between mares vs umbilical cord and foals for d-ROMs, and between mares vs foals for BAP. Pearson test showed a positive correlation between BAP and d-ROMs in mares (r= 0,644 p<0.05).

	Maternal blood	Cord blood	Foal blood	
d-ROMs (UCarr/l)	188.5±62.4a	143.3±61b	127.4±45.5b	P<0.05
BAP µmol/l	2063.9±459.7c	1772.1±485.9cd	1846.7±483.4d	P<0.05

Table 1. (a≠b; c≠d)

The concentration of oxidative agents has not been determined during pregnancy, but our results regarding the differences in OH concentration between mares and umbilical cord/foals at birth are in line to a previous study performed in women (Rogers et al., 1999, Lista et al, 2013). These differences could be due to the positive role of placenta in preventing the passage of oxidative agents from the mare to the foal, as supposed in human (Qanungo et al, 1999). Our results also show a parallel increase in antioxidative and oxidative parameters. This result may suggest a reaction in the antioxidative system secondary to an increased oxidative stress during critical period, such as parturition. Our results are in line a previous studies performed in cattle (Castillo et al, 2006; Albera and Kankofer, 2011). BAP concentration in the mare is higher than in umbilical cord and in the foal. The lower antioxidative activity in newborn foals can be explained by immature defence systems, as already reported for cows (Inanami, 1999; Albera and Kankofer, 2011).

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ANTIOXIDANT ACTIVITY OF HYALURONIC ACID INVESTIGATED BY MEANS OF CHEMILUMINESCENCE OF EQUINE NEUTROPHIL BURSTS AND ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY.

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The activated polymorphonuclear neutrophils (PMNs), the reactive oxygen and nitrogen species (ROS/RNS) released by PMNs and the derived inflammatory processes are strictly interlinked factors involved in the pathogenesis and progression of human inflammatory airways diseases such as chronic obstructive pulmonary disease (COPD), asthma and airway infections. Similar diseases are also present in horses which suffer from recurrent airway obstruction (RAO) and inflammatory airway disease (IAD). In order to decrease oxidative stress during inflammation and over-regulated PMN respiratory bursts, a rational strategy is to increase antioxidant defenses by administering agents with antioxidant activity. This approach offers more practical therapeutic possibilities and various antioxidant molecules are available. Hyaluronic acid, a naturally occurring high molecular weight non-sulfated glycosaminoglycan (GAG) composed of repeating disaccharide units containing glucuronic acid and N-acetylglucosamine, has numerous roles in the activation and modulation of the inflammatory processes, including the antioxidant activity. The aim of this study was to examine whether a preparation of hyaluronic acid (MW 900.000 Da) interferes with ROS and RNS during the course of equine PMN respiratory bursts.

To establish the lowest concentration at which this preparation of hyaluronic acid still has antioxidant activity, luminol amplified chemiluminescence (LACL) was applied. Electron paramagnetic resonance (EPR) spectroscopy was also used to investigate the direct antiradical (scavenger) activity of the preparation of hyaluronic acid.

The hydroxyl radical was significantly scavenged in a hyaluronic acid concentration-dependent way from 2.5 to 0.15 mg/ml. Superoxide anion, Tempol radical and the ABTS●+ were significantly inhibited from 2.5 to 0.62 mg/ml. Studying the LACL of stimulated equine neutrophils we observed that hyaluronic acid induced a statistically significant concentration-effect reduction of the LACL from 5 mg/ml to the last significant active concentration of 1.25 mg/ml. These findings were confirmed also when L-Arg was added in order to investigate the inhibition of the resulting peroxynitrite anion.

Our findings indicate that, in addition to the human use, hyaluronic acid can be adopted also for horses to antagonize the oxidative stress generated by free radicals. In order to maximize the therapeutic options a possible use in horse respiratory diseases through direct aerosol administration could be hypothesized in analogy with the same use in humans. This last possibility should be further evaluated.

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SUITABILITY OF FOUR DOG BREEDS AS CANINE BLOOD DONORS IN FOUR UNIVERSITY BLOOD BANKS

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to evaluate the prevalence of group DEA 1.1 blood in four dog breeds (Cane Corso, Bernese Mountain Dog, Retriever and crossbreed) selected as suitable blood donors by virtue of their size and nature and to assess reasons for exclusion from the canine blood donor program according to the Italian Ministry of Health Guideline on veterinary transfusion medicine (1).

retrospective analysis of data from four University Blood Banks (Milan, Perugia, Bologna, Pisa) belonging to the Italian Association of Veterinary Transfusion Medicine (AIMVET). Data recorded comprised signalment, nature, clinical evaluation, blood type (by card agglutination /immunochromatographic cartridge method), presence of infectious disease [Leishmania infantum, Ehrlichia canis, Rickettsia spp (IFAT), Babesia canis (IFAT/blood smear), Dirofilaria immitis (ELISA)], and reasons for exclusion from blood donor programs.

In total 212 dogs were evaluated (Perugia 93/212 (43.9%), Milan 74/212 (34.9%), Pisa 29/212 (13.7%), Bologna 16/212 (7.5 %)). Ages ranged from 2 to 8 years (median 4); 116/212 (54.7%) were female and 96/212 (45.3%) were male; weights ranged from 25 to 60 kg (median 34); 118/212 (55.7%) dogs were DEA 1.1 negative, and 94/212 (44.3%) were DEA 1.1 positive.
The largest group was the mixed-breeds (78/212, 36.8%), followed by Retrievers (68/212, 32.1%), Bernese Mountain dogs (41/212, 19.3%) and Cane Corsos (25/212, 11.8%).
In the mixed breed group prevalence of type DEA 1.1 negative blood was a 76.9% in Cane Corsos 76%, 42.7% in Retriever s and 24.4% in Bernese Mountain Dogs.
The reasons for exclusion from the blood donor program (15/212, 7.1%) were: positive test for infectious disease (10/15, 66.7%), poor venous access (4/15 dogs,26.7%), and unsuitable nature (1/15, 6.6%). In total 4.7% of the study population tested positive for an infectious disease.

the distribution of group DEA 1.1 blood in the evaluated population is similar to that reported in the literature.
Good venous access was reported in all evaluated breeds. Of the breeds examined in this study the Cane Corso group had a high prevalence of DEA 1.1 negative blood making it suitable for blood donation. Whilst there was a similar prevalence of DEA 1.1 negative dogs in the mixed breed group the quality of venous access was more variable in this group.
Retrievers and Bernese Mountain Dogs are suitable blood donors due to their size and nature but the higher prevalence of group DEA 1.1 positive animals in these groups restricts the use of their blood to DEA 1.1 positive recipients.

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EVALUATION OF GLUTHATIONE PEROXIDASE (GPX) IN CANINE CKD

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The aim of the study was to investigate the role of the Gluthatione Peroxidase (GPx) in the antioxidative metabolism in canine Chronic Kidney Disease (CKD.

A group of 40 dogs with CKD was classified into four subgroups of 10 subjects each by International Renal Interest Society (IRIS) system; a group of 10 healthy dogs formed the control group. Whole blood was sampled and collected in tubes containing lithium heparin, from each dog fasted for 12 hours, after a clinic examination. GPx was assessed by Ransel® test (Randox Laboratories Ltd Italia, TO) through Slim (SEAC, Calenzano, FI) spectrophotometer3. GPx values of different groups were compared by one-way ANOVA. GPx values were also analyzed using multifactorial ANOVA post hoc including the effects of some clinical parameters (weight loss, polyuria/polydipsia, vomiting). Linear regression test was also performed between GPx and serum creatinine.

GPx in whole blood was significantly different among IRIS groups ($p < 0.01$). GPx was significantly lower in group IRIS 1 (598±109 U.I.), compared to healthy subjects (1411±121 U.I.), with a decrease of 42,7%. GPx progressively increased in stage IRIS II (1196±152 U.I.), while it was similar to healthy subjects in group IRIS III (1329±204 U.I.). In IRIS IV an exponential increase of GPx (1841±216 U.I.) was reported. None of the symptoms was significantly correlated to GPx levels. A moderate positive correlation between creatinine and GPx was found ($r = 0,54$; $p<0,01$.

Some studies about GPx concentration in CKD have been conducted in both veterinary and human medicine. Most of them showed a progressive reduction in plasma GPx and an increased concentration of erythrocyte GPx in CKD patients, compared to control ones. Zachara et al., instead, reported a decreased concentration of erythrocyte GPx levels in the first stages of CKD1,2,5. The reduction of GPx in stage IRIS I, despite its later increase, could be explained by an initial consumption, followed by stimulation of its concentration, as a consequence of an excess of ROS. As GPx concentration reduces significantly in the first stage of CKD (when creatinine is still within reference range) and increases in the terminal phase of the disease, GPx could be helpful as diagnostic and prognostic marker of CKD. Although GPx can protect cellular components from oxidative stress by reducing the quantity of peroxides, when in excess it can cause a lack of minimal amount of oxidizing substances, which are essential for cell survival4. A larger number of subjects and further studies are required to explain better the role of GPx in the early diagnosis and prognosis of CKD, and to establish the dose of antioxidants to supply.

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ALOPERIDOL-INDUCED EXTRAPYRAMIDAL SIGNS IN THE HORSE: ONE CASE REPORT

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The extrapyramidal system is a neural network that helps in regulating and modulating motion. Extrapyramidal disorders lead to various movement inabilities, such as involuntary muscle spasms and motor restlessness with pacing and rocking without control. In equine practice, extrapyramidal signs (EPS) are relatively rare and the amount of involuntary motor activity, along with increased anxiety and agitation, is often confounded with seizures. Nigropallidal encephalomalacia, fumonisin toxicity, EPM, EHV-1, West nile virus (WNV) and rabies are classically reported as causes of EPS in the horse. Recently, several authors reported EPS in horses receiving phenothiazine sedatives such as fluphenazine decanoate. This acts by blocking dopamine receptors in the limbic region of the brain. The side effects are believed to originate primarily from the striatum, rich in D2 receptors. The aim of the present paper is to report a case of EPS possibly resulting from aloperidol administration, a butyrrophenone derivate that has pharmacological effects similar to phenothiazines.

A 10 y.o. warmblood mare was referred with sudden onset of seizure-like signs. The referring veterinarian reported that the horse received 5mg/kg aloperidol i.m. 5 days before admission. On arrival, the horse underwent a complete clinical examination, laboratory evaluation and both PCR and ELISA for EHV-1, EHV-4, WNV, Arterivirus, and Bornavirus. Initial treatment consisted in IV fluid therapy with ringer solution, dexamethasone, diazepam and ceftiofur.

The horse exhibited muscle fasciculations, compulsive and violent pawing and cycling, repetitive arching of the neck and knuckling on the forelimbs. Rectal temperature was 38.9°C, pulse rate 40 bpm and respiratory rate 12/min. Laboratory evaluation showed a stress leukogram and increased levels of glycemia, bilirubinaemia, AST, ALP, CK, LDH, lipase. Both PCR and ELISA were negative. Since the horse was not responsive to sedation after 3 consequent doses of diazepam, a 10 mg/kg intravenous phenobarbital grip was administered. This resulted in deep sedation and abatement of the abnormal behavior, and a maintenance therapy of phenobarbital (5 mg/kg per os q 12h) was started. Within the next 24 hours frequency and severity of episodes of abnormal behavior decreased progressively and fluid therapy was discontinued. On day 10 ceftiofur was discontinued. On day 24 the oral dose of phenobarbital was discontinued and, since no further neurologic signs were observed, the horse was discharged on day 30.

According to the history of the horse, clinical signs, response to therapy and lack of positive findings for other differential diagnoses, the diagnosis of aloperidol-induced EPS was made. The use of this dopamine antagonist was reported in the treatment of Equine Self Mutilation Syndrome. To the authors knowledge, no cases have been published previously describing EPS following administration of aloperidol in the horse.

ISOLATION AND CHARACTERIZATION OF PIG URINARY EXOSOMES FOR BIOMARKER DISCOVERY

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In recent years exosome research pubblications are rapidly expanding (1). These small vesicles (30-100 nm) of endocytic origin are thought to participate in cell—cell communication and protein and RNA delivery. A wide range of cells have been shown to release exosomes, but they have also been detected in several biological fluids, including plasma, urine, saliva and breast milk.
In particular urinary exosomes have been proposed as starting material to detect protein biomarkers of renal dysfunction and structural injury or overall to shed much insight on the health status of the kidney (2, 3, 4). We aimed to validate methods for exosome isolation from urine in a large animal model, the pig one. The swine shares with the man anatomical and physiological characteristics that make it preferred species as a pre-clinical model, in particular for kidney functions, surgical approaches and in the view to obtain a significant amount of biological specimens (urine and blood samples, tissue for renal biopsy) compared to other animals.

Exosomes were purified by differential ultracentrifugation, identified by electron microscopy and described in morphology, shape, size and distribution using atomic force microscopy (AFM). Validation methods include Western blot with pan-exosome markers and urinary specific exosome antibodies. Exosome protein content was analyzed through nanospray liquid chromatography-tandem mass spectrometry (LM-MS/MS). The total exosomal RNA purificated was evaluated using gel electrophoresis and a Bioanalyzer.

We found that the vesicles displayed a typical exosome-like size and morphology as analyzed by electron microscopy and AFM. Western blot and mass spectrometry further confirmed the presence of several exosome-associated molecules. Exosome RNA profile detected is typical of these vesicles, lacking rRNA subunits which are prominent in analysis of cellular RNA.

Characterization of isolated exosomes indicates that the described isolation methods are suitable for the subsequent RNA and protein profiling.

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PHARMACOKINETICS OF THE NOVEL ATYPICAL OPIOID TAPENTADOL AFTER INTRAVENOUS, INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION IN CATS

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The aim of the present research was to assess the pharmacokinetics of the novel atypical drug tapentadol (TAP) after intravenous (IV), intramuscular (IM) and subcutaneous (SC) injection of normal cats.

Six male and female mixed cats, aged 3-6 years, with a body weight (BW) of 3.4-4.8 kg, were used in a 3x3 Latin square cross over study. Each cat was administered with 5 mg/kg of tapentadol by the three administration routes. A catheter was placed into the right cephalic vein to facilitate blood sampling. Blood samples were collected at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 and 10 h after administration of TAP. The concentrations of TAP in plasma were evaluated using a previous validated HPLC method (1).

After IV administration some adverse effects including salivation, agitation and panting, were noticed in all the cats. However, they resolved rapidly (15-20 min) and spontaneously. These adverse effects were also detected after IM and SC administration but were less intense and of a shorter duration, and not in all the subjects (3/6 IM and 1/6 SC).

In all three administration groups, TAP concentrations were detectable in the plasma for up to 8 h. Figure 1 reports the average TAP plasma concentrations vs. time curves after the three administrations, respectively. After IM injection, TAP showed a variable but fast absorption (Tmax = 0.25 h, range 0.08-0.75 h) while after SC administration, absorption was significantly slower (Tmax = 0.63 h). The T1/2λz was quite similar between the three administrations routes in the range of about 2-3 h. Also VzF and ClF values were constant among the treatment groups. In the elimination phase of the curve, the decline of TAP was linear without any evidence of a secondary peak. The bioavailabilities (F%) were almost complete, accounting for 94% and 90% after IM and SC administrations, respectively. The plasma concentrations of TAP were over the minimal effective concentration reported in human beings for at least 3-4 hours after administration (Figure, window).

In conclusion this is the first study on the novel atypical opioid TAP in cats. There is some suggestion that TAP in the cat has different pharmacokinetic features as compared to other animal species (2,3). Although it would be premature to recommend the use of this drug in clinical practice (it would be ill-advised to administer a drug on the basis of pharmacokinetic data alone), this study could pave the road for further research on TAP in feline species. It is now critical to investigate both the analgesic efficacy and the actual effective plasma concentration to determine if TAP may be useful as an analgesic in cats. This could be particularly critical in animals sensitive to the adverse effects of commonly prescribed opioids, when they are experiencing moderate to severe pain.

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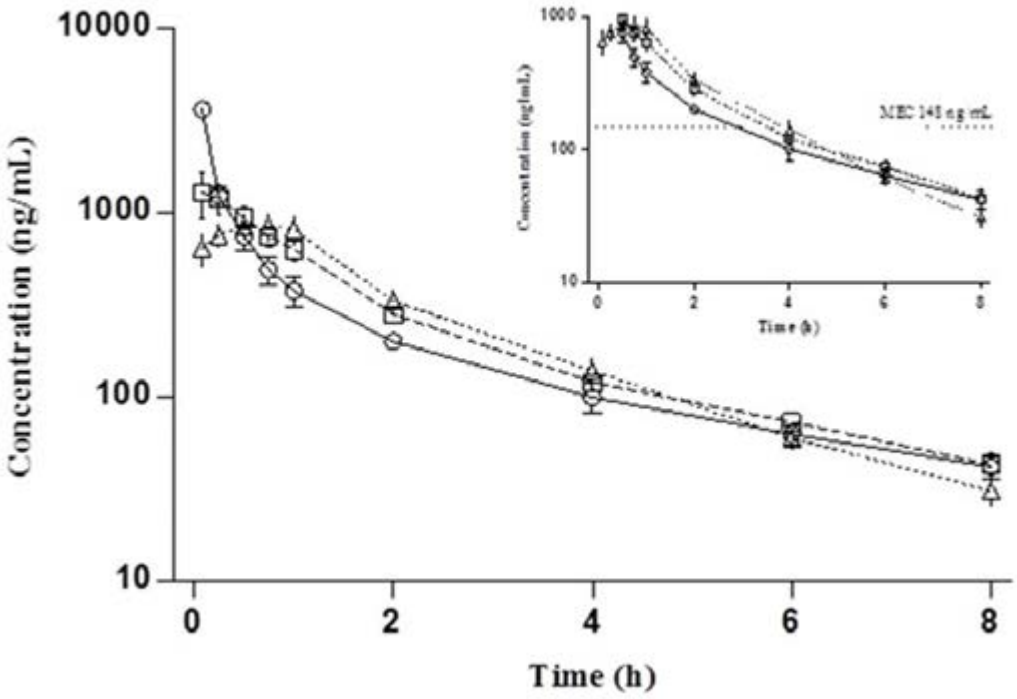
10000
1000
100
10
Concentration (ng/mL)
0
2
4
6
8
Time (h)

PHARMACOKINETIC FEATURES OF METOCHLOPRAMIDE IN RABBITS AFTER DIFFERENT ROUTES OF ADMINISTRATION

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To assess the pharmacokinetics (PK) of metochlopramide (MET) in rabbits after different routes of administration.

Six normal, male, white New Zealand rabbits weighing 3.0-3.5 kg were involved in the study. Animals were randomly assigned to four treatment groups, using an open, single-dose, 4-treatment, 4-period, unpaired, cross-over design (4x4 Latin-square). Animals in group I (n=2) received a single dose of 2 mg/kg of MET injected intravenously (IV) into the left marginal ear vein. Group II (n=2) received the same dose but by intramuscular (IM) route, injected in the middle quadrant of the buttock muscle. Group III (n=1), received the same dose via sub-cutaneous (SC) administration. Group IV (n=1), received a single dose of 4 mg/kg via per-rectum (PR) administration. The washout period was 1 week. The groups were rotated, changed in subject number and the administrations repeated. The blood (2 mL) was collected via indwelling catheter implanted in the central artery of the ear, at assigned times (0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4 and 6 hr). After four weeks, each rabbit had been administered with MET by the four routes.

The HPLC analyses were carried out according to Giorgi et al (1) shortly revalidated in rabbits. The PK analysis was carried out according to a non-compartmental (WinnonLin 5.3).

Followig IV administration the resulting plasma concentrations declined rapidly over the first hour and became undetectable after 4 hr. After the IM and SC treatments the plasma drug concentrations were below the LOQ of the method after 4 hr, while in the PR group only 2 out 6 animals showed detectable plasma concentration up to 4 h. The IM and SC administrations gave matching pharmacokinetic profiles, overlapping in the elimination phase with the IV curve (figure). MET was fastly absorbed (Tmax 0.19 min in both SC and IM groups, and 0.26 min in the PR group) and eliminated. All groups showed a short half life (0.81, 0,89 1.01 and 1.67 hours, in IM, SC, IV and PR administrations, respectively). The SC and IM bioavailabilities were high (112.0% and 96.2%, respectively), while the PR bioavailability was about 12.2%. This might be triggered by the sequestration of drug in fecal matter.

The results of the present study suggest that the IM and SC administrations of MET could be useful in treating gastrointestinal motility disorder in rabbits when a venous access is not available. The PR administration is likely to be unrealiable.

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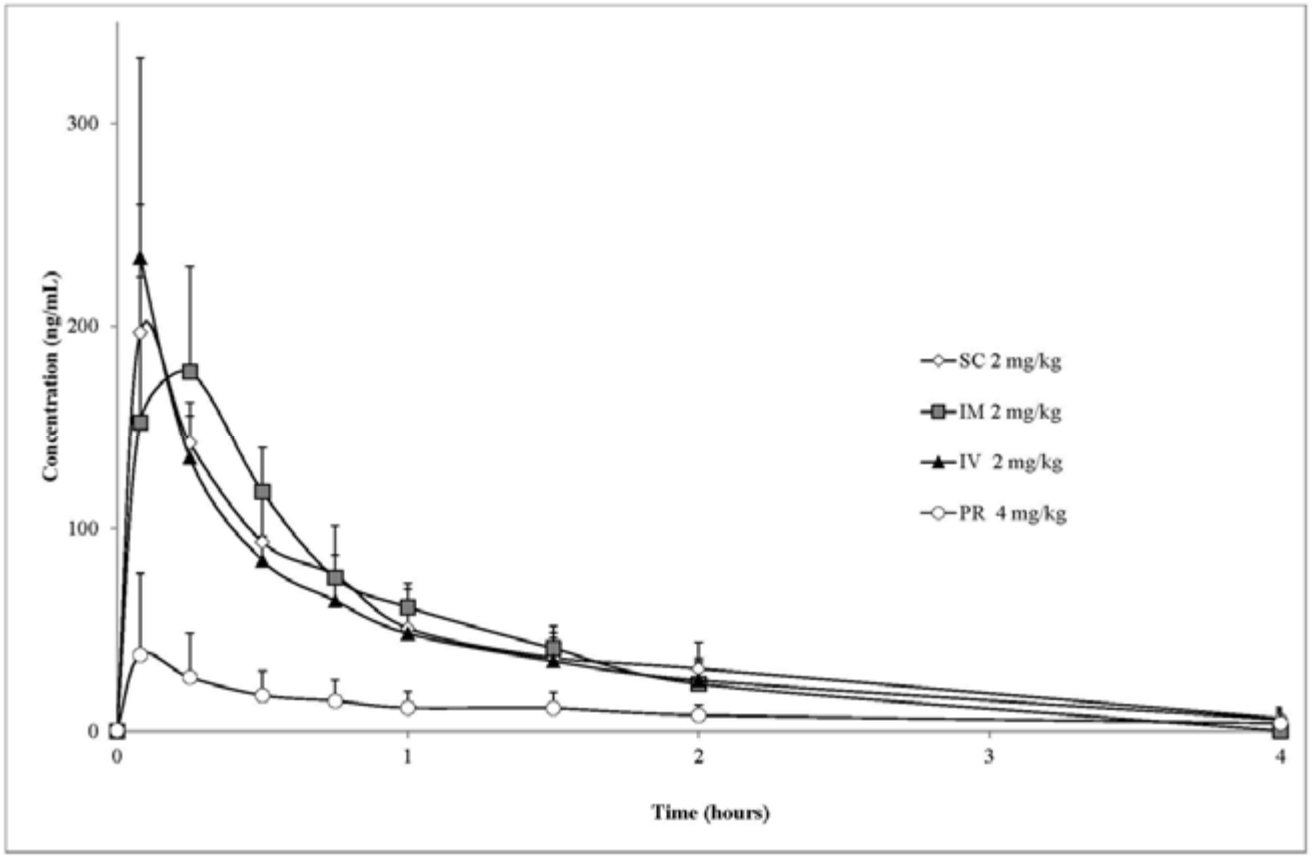
300
200
100
0
Concentration (ng/mL)
0
1
2
3
4
Time (hours)
SC 2 mg/kg
IM 2 mg/kg
IV 2 mg/kg
PR 4 mg/kg

EFFECTS OF TOPICAL ADMINISTRATION OF TIMOLOL GEL - FORMING ON INTRAOCULAR PRESSURE AND HEART RATE IN DOGS

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The goal of glaucoma management is to reduce intraocular pressure (IOP) and maintain it at a level compatible with the health of the optic nerve. New therapies are constantly being sought. To overcome the limitations of common eye drops, recent studies are developed a novel timolol mealate (TM) liposomal-hydrogel to enhance drug permeability and prolong residence time in the precorneal region, which achieved more effective local glaucomatous therapeutic effect.
The effects of topical administration of a single dose of timolol maleate 0.1% in gel-forming administered in the morning on intraocular pressure (IOP) and heart rate were investigate in 10 clinically normal dogs.

The drug was instilled in the both eyes once a day. The subjects received timolol in gel for 4 days, then, after a 10-day wash-out period, they received the placebo eye drops. Applanation tonometry and heart rate measurements were made at 8:00 AM, 16:00 PM and 24 PM daily during the 4 days of treatment and during the day that preceded treatment (adaptative phase).

Mean IOP was significantly reduced in the eyes treated with timolol when compared with the eyes treated with placebo. During the phase of treatment there is not daily fluctuations of IOP. All animals tolerate this topical medication well with no signs of discomfort, and no ocular side effects have been observed. A significantly reduction of heart rate was found in all subjects already in the second day of treatment.

Timolol 0.1% in gel-forming administered once day significantly reduces the IOP and daily fluctuations. So, as all B-Blocker drugs, induces a reduction of the heart rate.

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CLINICAL AND CLINICOPATHOLOGICAL FINDINGS ASSOCIATED TO TACROLIMUS ADMINISTRATION IN THE SWINE: PRELIMINARY RESULTS

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Tacrolimus (Tac), a calcineurin inhibitor, is widely used in immunosuppressive protocols for human organ transplantation and is reported to induce many side effects, including nephrotoxicity (1). Pigs are considered a good animal model for organ transplantation in human medicine, however studies on Tac treatment to experimental animals are limited (2). The aim of our preliminary study was to evaluate clinical and clinicopathological alterations associated with Tac administration in healthy pigs.

Three healthy female pigs (mean body weight 79,6±2,5 kg) were recruited and kidney biopsies were performed before, during and at the end of the experiment. Animals were followed for 4 weeks. Tac was given orally (PO) in 1 pig and by continuous rate infusion (CRI) in the other 2 ones using a jugular catheter. The drug administration was scheduled at incremental dosages of 0,5 mg/kg/week starting from 0,5 mg/kg/day for PO treatment and at incremental dosages of 0,05 mg/kg/week starting from 0,15 mg/kg/day for CRI. Complete blood count, serum biochemistry profile, Tac blood concentration and urinalysis were performed two times a week. Trial has been approved by ethics committee.

Clinical signs potentially associated with Tac administration appeared early (first week) and were more evident with high Tac blood concentrations (32,5 ng/ml for PO administration - 1,5 mg/kg/day; and 62,2 ng/ml for CRI - 0,25 mg/kg/day). The main clinical signs were dullness, reduced appetite, muscular and joint pain, fever and chills. With PO administration, the animal developed diarrhea and anorexia. To achieve the end of the experimental period animals needed treatment with antibiotics and fluid therapy. No significant alterations for clinicopathological variables were detected, but an increase respect baseline values was observed for urine specific gravity, creatinine and total protein concentration, while a decrease was noticed for albumin concentration, albumin to globulins ratio, hematocrit value and white blood cell count, in particular for total lymphocytes. At the time of euthanasia renal biopsies showed only mild signs of renal damage characterized by interstitial inflammation and glomerulopathy.

The increase of Tac blood concentration in swine is associated to relevant clinical side effects without significant clinicopathological abnormalities in a 4 week period. Our findings are suggestive of progressive dehydration associated to systemic inflammation probably related to immunodeficiency. No clinical evidence of nephrotoxicity was documented. Further investigations are needed to better identify Tac dose that induce kidney toxicity in swine.

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IGG LEVELS IN THE FOAL: COMPARISON OF TWO METHODS

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The Failure of Passive Transfer (FPT) is a predisposing factor for severe disease states. The aim of this study was to compare two methods for determination of serum IgG.

Fifteen foals, 6 fillies e 9 colts weighed 47-52 Kg, were included in the present work. APGAR score was evaluated at 5 minutes after delivery (Martens, 1982) and semeiological parameters were recorded (Stoneham, 2008). For each foal, blood sample was collected from the jugular vein in EDTA tubes at birth and in serum tubes at 24 hours of age. EDTA samples were used for total WBC count and N:L (Axon and Palmer, 2008) within 2 hours after collection, while serum was used to determine IgG concentration by two methods: 1) a semiquantitative method (ELISA test, Snap Foal IgG Test Kit) and 2) a quantitative method (colorimetric fothometric method, DVM Equine Serum IgG Test Analyser, Labstock Microservices, TM VDx Inc. Belgium). Foals were divided retrospectively in two groups on the basis of IgG levels (IgG Snap foal, Idexx, USA): Group A: good transfer of passive immunity (IgG>800 mg/dl), group B: partial/failure of passive transfer (IgG<800 mg/dl) (Vaala, 1994)

Group A: 11/15 (5/11, 45.4% fillies and 6/11, 54.5% colts) foals were included in this group. All the foals were born from normal delivery, presented an APGAR score >7, semeiological data within normal range, and N:L>2. Group B: 4/11 (3/4, 75% colts and 1/4, 25% fillies) foals with FPT were included in this group. One/4 foal was premature (gestational length < 330 days and N/L 0,15), presented pathological semeiological parameters (quadrupedal standing after 240 minutes and first suckling after 300 minutes after birth); 3/4 foals were born from normal delivery and after a physiological gestation, APGAR score was >7, semeiological parameters were within normal range and N:L was >2. In all the foals included in group B, IgG levels evaluated with semiquantitative method were between 400 and 800 mg/dl (partial failure), while IgG levels obtained with quantitative method showed a complete failure in 1/4 foal and a partial failure in 3/4 foals.

The quantitative method seems a better method to determine IgG levels than the semiquantitative one, because it can dose exacly the IgG concentration and it is not affected by the operator.

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PHARMACOKINETIC PROFILES OF CIMICOXIB

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This study was performed to evaluate the pharmacokinetic (PK) profiles of cimicoxib (CX), a novel cox-2 inhibitor, in dogs

The study protocol was approved by the animal welfare committee of the Lublin University. Six healthy male Labrador Retriever dogs weighing 32-41 kg and ranging in age between 2-4 years were used. Animals were randomly (drawn from a box) assigned to two treatment groups, using an open, paired, single-dose, two treatment, two-period, crossover design. Each subject received a single dose of 80 mg/subject CX (Cimalgex®, Vetoquinol). The first group (n = 3) received the treatment in the morning, after fasting for 12 h overnight. The second group (n = 3) was treated with a single dose of 80 mg/subject CX in the morning, 15 min after the dogs had been fed. Canned meat dog food (250 g) with oatmeal (200 g) in a 20 cm squared container. After a wash-out period of 2 weeks the groups were reversed and the treatments repeated. A catheter was placed into the right cephalic vein to facilitate blood sampling. Blood samples for PK analysis (2 mL) were collected at intervals of 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, and 24 h after CX administration, and placed in collection tubes containing lithium heparin. The blood samples were centrifuged at 400 g for 5 min within 30 min of collection. The harvested plasma was stored at -20 °C and used within 14 days of collection. The plasma concentrations of CX were detected by a validated HPLC method (1) and PK analysis performed by Winnolin 5.3 software.

No adverse effects were observed in any dogs during the experiment. CX concentrations were detectable in plasma for up to 24 h in 3 out of 6 dogs in both the groups. Overall the mean PK curves for fasted and fed status showed similar but not identical (Figure 1). Four dogs showed individual PK profiles that were almost identical in fasted and fed state, while two subjects demonstrated variations in their PK profiles that were influenced by fasted/fed status. The AUC0-∞ in both fasted and fed treatments was similar (P=0.50). The mean value of Tmax and absorption half-life in the fed group was slightly delayed compared to the fasted group and Cmax was slightly decreased in the fed group (P = 0.74). However, these small differences between groups were not significant. The rate of CX absorption remained almost unchanged regardless of fed or fasted state.
Large differences have been found in both intra- and inter subject PK profiles. The CYP polymorphism (CYP2D6 and CYP2C19) already detected in Beagle dogs (2) might also have affected the dogs used in the present study explaining the inter-subject differences.

There were no significant differences in PK values between the fasted and fed condition. However, the factors that evoked the intra-subject differences in 2 dogs (both in fasted and fed states) are obscure and further studies will need to clarify this issue.

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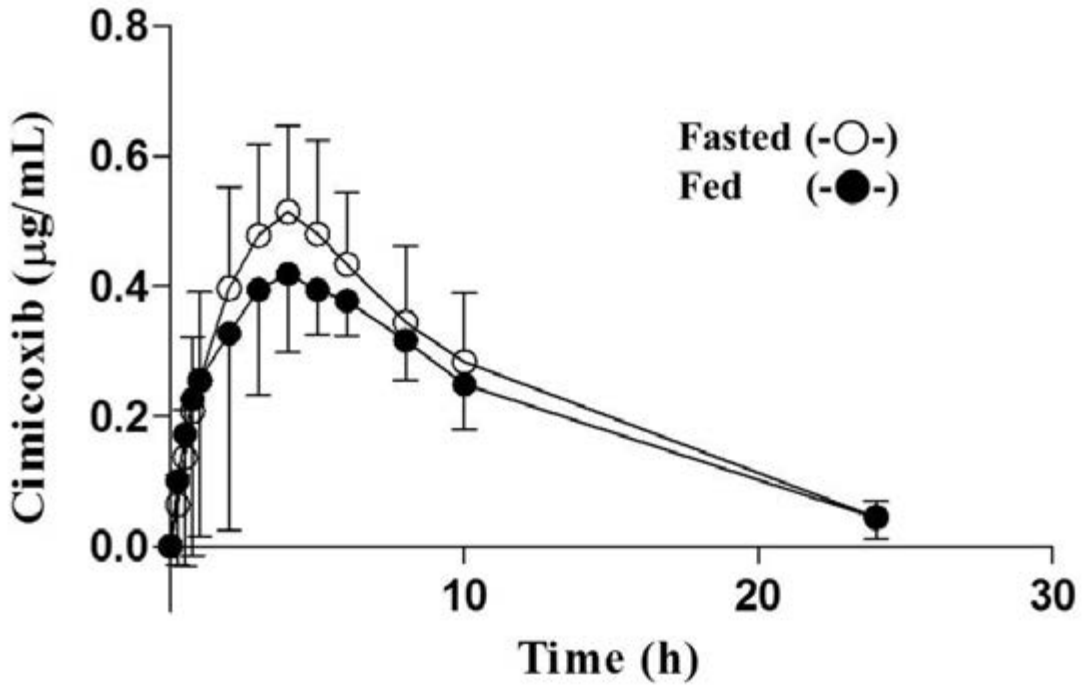
0.8
0.6
0.4
0.2
0.0
Cimicoxib (µg/mL)
10
20
30
Time (h)
Fasted (-○-)
Fed (-●-)

INFLUENCE OF DIFFERENT LEVELS OF POSITIVE INSPIRATORY PRESSURE ON PULMONARY VENTILATION IN DOGS: A COMPUTED TOMOGRAPHY STUDY

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Thoracic computed tomography in small animals is usually performed with the patients in apnea and ventilated with a constant positive inspiratory pressure (breath hold technique), in order to the reduce the interference of movement and pulmonary atelectasis (1, 2). The aim of the study was to compare pulmonary ventilation at different levels of PIP in dogs under general anesthesia.

Sixty dogs scheduled for thoracic computed tomography for the evaluation of pulmonary metastatic masses, were included in this study. All patients were anesthetized with a standard protocol, orotrachealy intubated and connected to a pulmonary ventilator (Siemens 900 D) and ventilated in a volume controlled mode. Frontal topograms and helical CT of the chest were obtained with a third generation spiral TC (GE ProSpeed sx®,General Electric, USA), at end-inspiration apnea with the patients positioned in the scanner in dorsal recumbency. The CT scan was performed after apnoea was induced by the administration of 2 µg/kg of fentanyl IV followed by the administration of a constant positive airway pressure (CPAP) The level of CPAP was randomly applied at 6 different levels : PIP 0 , PIP 5, PIP ,PIP 10, PIP 12 and PIP 15 cmH2O. Mechanical ventilation was resumed immediately after the CT images were obtained. The CT images were analyzed by means of a computer program (DicomWorks v 1.3.5.). In accordance with previous studies (1), we identified the following aeration regions or compartments within the lung: hyperinflated (– 1,000 to – 901 HUs), normally-aerated (900 to -501 HUs), poorly-aerated (-500 to -101 HUs), and non-aerated (-100 to + 100 HUs). Numeric surface area values of each compartment were expressed as a percentage of the total lung surface.
Data regarding lung aeration (% of hyperinflated, normally aerated, poorly aerated and atelectasic lung parenchyma) at each PIP level were compared with data obtained at PIP 0.

The % of atelectatic lung area was higher in PIP10 , PIP12 and PIP15 as compared with PIP 0. The % of the poorly-aerated lung area was lower in PIP8 ,PIP10 ,PIP12 and PIP15 as compared with PIP0 .The % of normally aerated lung area was higher in PIP10, PIP12 and PIP15 as compared with PIP 0 .The % of hyperinflated lung area was higher at PIP15 as compared with PIP0 .

The results of this study demonstrated that a positive inspiratory pressure between 10 and 12 cmH2O during the breath hold technique can minimize the affects of anesthesia on pulmonary ventilation, improving the diagnostic quality of the tomographic images.

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THE USE OF TRAMADOL IN IMPROVING THE EFFICACY OF KETAMINE–MEDETOMIDINE ANAESTHESIA IN PIGS UNDERGOING CASTRATION

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To evaluate whether the use of tramadol reduces the intraoperative responses to elective castration and postoperative pain in pigs.

Ten 40 day old male pigs were included in the study and a blood sample was taken to evaluate baseline cortisol concentration (T0). The animals were randomly divided into two groups. The first group was premedicated IM with 5 mg/kg of tramadol, 0.01 mg/kg of medetomidine and 10 mg/kg of ketamine (treatment KMT); the second group received the same dose of medetomidine and ketamine mixed with an equal volume of normal saline (treatment KMS). Anaesthesia was induced IV with propofol to effect (2 mg/kg) and the trachea was intubated. The anaesthesia was maintained with isoflorane in 100% oxygen by the to-and-fro anaesthetic breathing system. All the animals were castrated and during the surgery heart rate (HR), respiratory rate (RR), body temperature (T), percentage oxygen saturation (SpO2), expiratory carbon dioxide concentration (EtCO2), inspired oxygen concentration (FiO2), inspiratory and expiratory isofluorane concentration (FiIso and EtIso) were recorded every 5 minutes. Blood samples were taken from all the pigs to evaluate cortisol concentration as follows: when the animals assumed lateral recumbency (T1), when the spermatic cord was ligated (T2), when the animals achieved recovery box (T3), and 30 minutes later (T4). T-Student test was used to analyze the data and differences were considered significant if $p < 0.05$.

Both treatments provided smooth induction, good immobilisation, and adequate anaesthesia to perform the surgery. There were no significant differences in intraoperative parameters between the two treatments except in the HR values and in the EtIso, which were significantly lower in the KMT treatment ($p< 0.05$) compared to the KMS treatment. No differences were found in the basal and intraoperative cortisol value ($p\geq0.05$) of each group. However, the T4 sample showed a statistically significant decrease in the KMT treatment ($p< 0.05$) compared to the KMS treatment.

Although both treatments were appropriate for routine pig castration, the use of tramadol in the KMT treatment decreased the pigs' intraoperative response to nociception induced by castration, witnessed by HR and EtIso, which were lower than the KTS treatment. Furthermore, the analgesia in the postoperative period was better in the KMT treatment, as shown by the T4 sample, which was lower than the KMS treatment. The addition of tramadol to the anaesthetic protocol in the KMT treatment improved the analgesia reducing the intra-operative pain associated with pig castration, without causing any collateral effects. Moreover, tramadol increased the duration of antinociception in the post-operative period without increasing the duration of anaesthesia, nor causing additional depression of the cardiorespiratory parameters.

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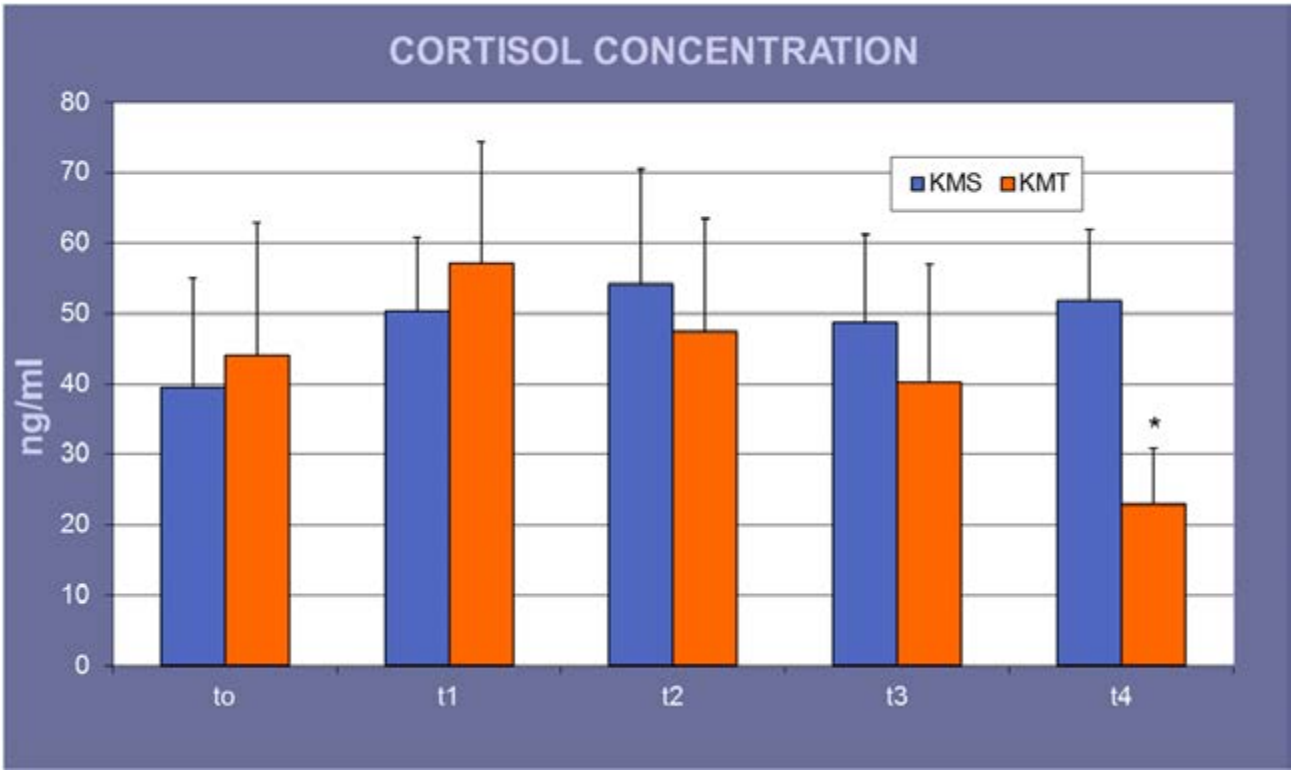
CORTISOL CONCENTRATION
ng/ml
80
70
60
50
40
30
20
10
0
KMS
KMT
*
to
t1
t2
t3
t4

DETERMINATION OF MINIMUM ALVEOLAR CONCENTRATION (MAC) OF SEVOFLURANE IN MECHANICALLY VENTILATED SHEEP.

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To determine the minimum alveolar concentration (MAC) of sevoflurane in mechanically ventilated sheep.

Prospective study. Fourteen healthy adult female Sardinian bred sheep were selected. Anaesthesia was induced with sevoflurane delivered in oxygen (3 l/min) through a mask with the vaporizer set at 8%. An endotracheal tube was inserted, and a probe for continuous measurement of end-tidal and inspired sevoflurane concentrations was placed in the trachea via the endotracheal tube. After 30 minutes of equilibration at an end-tidal sevoflurane concentration of 2,8%, an electrical stimulus [5Hz /1 msec (impulse duration) / 50.0 mA] was applied to the skin of the lateral side of the right antebrachium for one minute or until a gross purposeful movement response was obtained. The vaporizer dial setting was changed in order to obtain an increase or decrease by 0,1- 0,2% in end-tidal sevoflurane concentration, dependent upon whether a motor response had been elicited by noxious (electrical) stimulation or not. Following a 10-minute equilibration period the noxious stimulation was repeated. The MAC was defined as the mean of the lowest end-tidal sevoflurane concentration that prevented a positive motor response and the highest concentration that allowed a positive motor response and determined always twice. Time to induction of anaesthesia, time to extubation and time for recovery (i.e. time to standing up) were also recorded.

The mean± SD of the MAC of sevoflurane was 2,6±0,3%. Time to intubation was 223±106 seconds, time to extubation was 351±121 seconds and time to recovery was 780±282 seconds.

Sevoflurane administration in sheep allows for a smooth induction of anaesthesia and fast extubation and smooth recovery from anaesthesia. The MAC of sevoflurane measured in sheep was slightly higher than that reported in other species (e.g. goat and llamas).

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INTRANASAL (IN) ADMINISTRATION OF DEXMEDETOMIDINE AND BUTORPHANOL: A PRELIMINARY STUDY IN DOGS

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Aim of this study was to assess in dogs the sedative effects of the intranasal(IN)administration of Dexmedetomidine alone or combined with Butorphanol.

N. 20 dogs of different breeds, ASA I or II, undergoing sedation were included in the study. Written consent was obtained by the owners. Baseline measurements of T°C, RR, HR, NIBP and SPO2 were recorded noninvasively (Mindray PM-9000 Express®) at T-0 and every 5 minutes after treatment. Dogs were then randomly assigned to one of two groups receiving either Dexmedetomidine (Dexdomitor® Pfizer 20 µg/kg = group D) or Dexmedetomidine (10 µg/kg) mixed with Butorphanol (Dolorex® Intervet 0.1 mg/kg = group DB). The drug/s were administered IN by a Mucosal Atomization Device (MAD® Wolfe Tory Medical, inc.). A blinded observer assessed onset, duration and degree of sedation every 5 minutes using a numerical scoring system (maximum sedation score [SS] = 10). Total SS at each time point of the two groups were compared using Pearson's χ2. Clinical parameters were processed by repeated measures MANOVAs followed by post hoc Tukey's HSD test to explore differences between single time points ($P \leq 0.05$).

IN drug absorption was excellent and comparable to IM injection in all cases (onset = 12.6'± 5'). Total SS was significantly higher in Group D (mode 9) compared to group DB (mode 8) ($P=0.004$). Mean blood pressure was significantly higher in Group D at T15, T20, and from T30 to T45 ($P \leq 0.01$). No significant differences in SPO2, HR and RR were found between the two groups at all times after premedication. No undesirable effects were observed in any of the dogs.

IN administration of sedative drugs is a widespread procedure in human adults and children and an attractive target in animals. A direct drug transport pathway from the nasal cavity to the brain has been shown (1). Nasal administration of diazepam has been reported in dogs as means of emergency treatment for seizures, resulting in a rapid and efficient absorption (2). Intranasal dexmedetomidine and opioids have been reported for sedation in children (3, 4) along with reversal agents. The use of the MAD® device improves drug distribution on mucous membranes, resulting in an easy, efficient and painless administration procedure (4). The present study endorse IN administration of Dexmedetomidine and Butorphanol for routine clinical use in dogs.

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COMPARATIVE STUDY ON THE ANALGESICS EFFECTS OF FLUNIXIN MEGLUMINE AND INTRAFUNICULAR LIDOCAINE FOR THE MANAGEMENT OF POSTOPERATIVE PAIN IN LAMBS CASTRATION

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Although lambs castration is a common husbandry procedure, it's often associated with short and long term pain (1). The aim of this study was to evaluate, in a population of lambs castrated in GA, the effects of intrafunicular Lidocaine and systemic Flunixin, alone or in association, on intra and postoperative pain and distress indicators for 12 hours after surgery.

30 homogeneous male lambs, 90 days-old, were randomly assigned to the following 5 treatments: C (sham manipulation of the testis during GA) - O (standardized orchiectomy under GA) – F (Flunixin 1,1 mg/kg e.v. 1 h before castration and SID for 3 days) – L (Lidocaine 3 mg/kg + 10µg of adrenalin in 7 ml solution injected in each funiculus just before castration) - FL (intrafunicolar Lidocaine and Flunixin treatments administered before castration).
Heart and respiratory rate (HR, RR) and rectal temperature (RT) were registered for 5 days after surgery. During orchiectomy, systolic, diastolic and mean arterial blood pressure (SAP, DAP, MAP), inspiratory and expiratory concentrations of isoflorane (ISO), CO2 and O2, FiO2, HR, RR and eye reflex were recorded every 5' for 7 times (Ta1-Ta7, being T3 the skin incision time). Individual plasma cortisol concentration (PCC) was evaluated before surgery (Tc0-basal) and 30'-90'-180'-360'-540' after surgery (Tc1-Tc5). Blood glucose was measured using reactive glucose test strips, before surgery (Tg0) and 120'-240'-360'-720' after surgery (Tg1-Tg4). Blood samples for complete hematology were collected before surgery (Th0) and 24, 48 and 72 hours after treatments (Th1-Th3). Lambs behavior was recorded for 12 hours after surgery by a digital camera (2). Statistical analysis was performed using a GLM Anova.

Lambs receiving intrafunicular Lidocaine experienced acute pain soon after the injection, during surgery, as shown by intraoperative HR, RR and MAP. This can be due to the volume used, the acidic pH solution, or the effect of adrenaline despite the same increase was not evident in group FL (3). In group L the PCC peak lasted considerably longer compared to other groups (table 1), showing persistent postoperative distress.
RR and HR in all lambs were higher on day 1 compared to days 2-3-4-5 and pain avoidance behaviors were detected in all groups, expect C, since the 1st hour after surgery; all lambs castrated, especially group O, showed prolonged ventral recumbency, indicative that castration caused pain. A less sustained PCC suggested that lambs receiving the association Flunixin/Lidocaine experienced the least pain in the postoperative period.

In accordance to previous studies in conscious animals, systemic NSAIDs combined with intrafunicular anaesthesia can be recommended to provide intra and postoperative analgesia during routine castration in lambs.

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	0	F	C	FL	L
Tc0/0	25.6	38.2	23.8	18.6	22.0
Tc1/0,5h	106.2^{A}	98.1^{AB}	40.8^{C}	64.9^{BC}	95.4^{AB}
Tc2/1,5h	85.9^{A}	58.3^{AB}	27.1^{B}	23.3^{B}	82.3^{A}
Tc3/3h	81.1^{AB}	52.3^{BC}	28.8^{C}	17.2^{C}	87.2^{A}
Tc4/6h	49.4^{ab}	31.1^{b}	27.1^{b}	19.9^{b}	83.9^{a}
Tc5/9h	43.0	28.9	26.4	14.0	41.2

METHADONE AND DEXMEDETOMIDINE COMBINATION AS PREMEDICANT FOR OVARIECTOMY AND ORCHIECTOMY IN CATS

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The aim of the study was to evaluate sedation level and analgesic efficacy produced by the association of methadone 0.5 mg/kg and dexmedetomidine 5 mcg/kg for neutering surgery in cats.

Thirty client-owned cats (8 males and 22 females) anaesthetised for elective neutering surgery, considered healthy on the basis of clinical exam and haematological tests, were enrolled in the study. Cats were premedicated with dexmedetomidine 5 mcg/kg and methadone 0.5 mg/kg administered intramuscularly. Cats were then induced with propofol, intubated and maintained with isoflurane in 100% oxygen. Time to reach the sedative effects wasregistered as well as the following clinical parameters: heart rate (HR), systolic arterial pressure, using the doppler technique (SAP), respiration rate (RR), end tidal CO2 (EtCO2), haemoglobin oxygen saturation (SpO2), temperature (T°) and end tidal isoflurane percentage (EtIso). All the parameters were recorded every five minutes until the end of anaesthesia. Three different simple descriptive four points scales were used to evaluate the degree of sedation after premedication, the catheter placement and the quality of recovery after general anaesthesia (Bortolami et.al 2011), starting from 0 (no sedation, difficult catheter placement, poor recovery) to 3 (profound sedation, very easy catheter placement, excellent recovery).

Mean time to reach the maximum sedation level was 4 ± 5 minutes; median sedation score resulted 2 (0-3), median catheter placement score was 3 (0-3) and median recovery quality score was 3 (0-3). Mean dosage of propofol for intubation resulted 1.6 ± 1 mg/kg. EtIso during the surgery variated from 0.83 to 1.4 %. Nine of the 30 cats (30%) in the study required fentanyl rescue analgesia during the surgery; one cat received dopamine infusion as hypotension treatment; 6 cats received assisted mechanical ventilation and 1 cat received controlled mechanical ventilation.

The association of dexmedetomidine 5 mcg/kg and methadone 0.5 mg/kg resulted in an overall good quality sedation with some exception: in fact 3 cats presented a poor sedation score (0-1), nevertheless the score for the catheter placement was always 2 or more. The onset of the sedation was quite fast and the association of drugs resulted in propofol dose-sparing effect. A high percentage of patients required rescue pain management during surgery showing inadequate analgesia in those cases.
In conclusion premedication with methadone 0.5 mg/kg and dexmedetomidine 5 mcg/kg given intramuscularly, resulted in a good combination drug protocol which allow to handle cats easily and the use of low-dose propofol for anaesthesia induction; however the analgesia obtained was not always adequate.

Bortolami E, Murrell JC, Slingsby LS. Methadone in combination with acepromazine as premedication prior to neutering in the cat. Vet Anaesth Analg. 2013 Mar;40(2):181-93.

TARGET CONTROLLED INFUSION (TCI) WITH PROPOFOL: APPLICATION IN DOG - A PRELIMINARY STUDY

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Propofol is an anesthetic agent used in totally intravenous anesthesia (TIVA) and target controlled infusion (TCI). The aims of this work are of various nature. First of all, the applicability of TCI total controled infusion on dog by using a syringe pump, realized for Human Medicine, with the purpose to define adaptability criteria between man-dog parameters. Further various different concentrations of Propofol effectors' site were considered to detect the ones that can better ensure a suitable anesthesiology plan as well as a stable hemodynamic and respiratory situation during surgery. To end to find the actual Propofol blood plasma concentrations and then they have been compared with the data measured by the syringe pump. In that way, the results could be correlated to the clinical data recorded during surgery.

This prospective study covers 9 ovariectomized dogs in general anesthesia of 60-90 minutes. The enrolled dogs had a weight exceeding 15 kg, and normal laboratory tests results. After premedication with acepromazine (10 µg/kg IV) followed, after 5 min, by the administration of fentanyl (load bolus 2 µg/kg IV + CRI (Constant Infusion Rate) 5-10 mg/kg/h IV), the animals were induced and maintained with Propofol using a Syringe Pump Terfusion® 372 TCI/TIVA (Terumo). The target concentrations of Propofol ranged from 5 µg/ml to 10 µg/ml. On the basis of concentrations established previously with TIVA technique, in the first animal the target concentration was set at 5 µg/ml, but it was necessary to increase the rate of infusion during surgery. In two animals was set at 7 µg/ml, in four at 8 µg/ml and in the last two animals at 10 µg/ml. Blood samples were collected, from a catheter positioned in a cephalic vein, before treatment, at established intervals (2, 5, 10, 15, 20, 25, 35, 45, 55 and 60 min), and after any possible change of the rate of infusion. At the end point of the Propofol administration, blood samples have been taken at time 2, 5, 10, 15, 25, 35 and 45. After blood centrifugation the plasma was collected and stored at -20 °C until analysis for propofol concentration measured with a HPLC technique. Each dog's clinical/monitoring parameters and the target and predicted concentration were recorded at each sampling time.

In most cases, the plasmatic concentration of Propofol proved to be correlate to the variations of the clinical parameters. A tendency to under-dosing Propofol plasma concentration, compared to those estimated by the infusion pump, was also noted.

This study allowed to introduce into the Veterinary anesthesiology practice a new device to achieve TIVA and TCI. Furthermore, it has resulted in a preliminary TCI protocol applied in conjunction with major veterinary surgery.

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USE OF NANOHYDROXYPATITE IN REGENERATIVE THERAPY IN DOGS AFFECTED BY PERIODONTOPATHY: PRELIMINARY RESULTS

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Nanosized ceramics may represent a promising class of bone graft substitutes due to their improved osseointegrative and osseoinductive properties. Nanohydroxyapatite binds itself to the bone and favours bone healing by stimulation of osteoblast activity (Singh et al., 2012). The present study aims to analyse the in vivo behaviour of nanohydroxyapatite and to assess its regenerative capacity in dogs affected by periodontal disease.

Twenty-eight dogs of different breeds, aged between 5 and 15 years, were employed in the study and were randomly subdivided into a control group and an experimental group. After clinical, instrumental and radiological examinations to estimate the severity of the disease, all the subjects underwent dental prophylaxis and a bioptic sample was taken. A histopathological examination of the periodontal tissues, in correspondence with teeth with periodontopathy ranging between grades II and III, followed. Regenerative therapy with applications of nanohydroxyapatite was administered only to the dogs of the experimental group. After a period of between 30 and 42 days, a further clinical, instrumental and radiological examination was carried out and a bioptic sample taken solely on the dogs whose histological examinations showed changes ascribable to periodontal disease.

The results of the histopathological examination demonstrated that the subjects belonging to the control group, who only underwent the dental prophylaxis, in no case showed any histopathological improvement. In 6 out of 14 cases, the situation remained stationary and in the remaining 8 there was a clear deterioration. On the contrary, all the dogs in the experimental group, who underwent dental prophylaxis together with the administration of nanohydroxyapatite, showed clear signs of improvement with respect to their initial condition. Furthermore, there was no sign of any inflammatory reaction in the areas which had been treated with nanohydroxyapatite.

In conclusion the study demonstrated the regenerative potential of nanohydroxyapatite in periodontal therapy. In fact, its use as a graft material has produced very satisfactory results, which have been supported without doubt by the histopathological examinations. Thus, nanohydroxyapatite represents a valid osteoconductive and osteoinductive graft product in dogs. However, more research is needed and it is, therefore, imperative to extend the case histories and further standardize diagnostic methods.

Singh VP, Nayak DG, Uppoor AS, Shah D: Clinical and radiographic evaluation of Nano-crystalline hydroxyapatite bone graft (Sybograf) in combination with bioresorbable collagen membrane (Periocol) in periodontal intrabony defects. Dental Research Journal, 2012; 9: 60-67.

HIND LIMB ALIGNMENT IN TOY BREED DOGS: COMPARISON BETWEEN SUBJECTS AFFECTED AND FREE FROM MEDIAL PATELLA LUXATION

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Medial patellar luxation (MPL), described as the most common orthopaedic disease affecting the canine stifle. MPL has been recognised as the most common congenital pathology (7.2%) in immature dogs. Small-breed dogs are 12 times more likely to develop MPL than large breed dogs. The cause of MPL remains unclear. Our objectives were 1) to report anatomic and mechanical joint angles of the femur and tibia in small and toy breed dogs free from patella luxation to develop reference ranges and 2) to compare this data to values of toy and small breed dogs affected by patella luxation.

Digital images of the pelvis, femora and tibia of toy and small breed dogs were prospectivelly obtained for measurement. Hindlimbs were divided in five groups, according to a grading system based on the findings at physical examination: 1) free from patella luxation, 2) affected by I° of patella luxation, 3) affected by II° of patella luxation, 4) affected by III° of patella luxation, 5) affected by IV° of patella luxation. Data obtained included: the inclination angle (ICA), anatomical lateral distal femoral angle (aLDFA), mechanical lateral distal femoral angle (mLDFA), mechanical lateral proximal femoral angle (mLPFA), anatomical lateral proximal femoral angle (aLPFA), femoral anteversion angle, tibial plateau angle (TPA), mechanical cranial proximal tibial angle (mCrPTA), mechanical caudal proximal tibial angle (mCaPTA), mechanical medial proximal tibial angle (mMPTA) and mechanical medial distal tibial angle (mMDTA) in toy breed dogs (TBD) with and without MPL. Data from each group were combined and means ± SD were determined. A t-test was performed to assess significant differences for all outcome measures between cases free from patella luxation and cases affected by patella luxation. A t-test was performed to assess significant differences for all outcome measures between groups 1, 2, 3, 4, 5. Significance was set at $P<.05$.

Stifles of forty eight TBD (weight less than 7.5 Kg) were graded and allocated into five group. Data are reported in Table I and Table II. Patients affected by IV° of patella luxation (group 5) were significantly different in relation to aLDFA and mMPTA.

These data provide reference values for tibial and femoral joint angles for toy and small breed dogs. These values will be useful to surgeons for determining whether or not angular deformity of the femur is present and aid in determining the degree of correction necessary to restore alignment. In our cases, aLDFA and mMPTA were significantly higher ($p<0.05$) in patients affected by patella luxation in respect to dogs free from patella luxation. Higher values of aLDFA and mMPTA explain the predisposition for medial patella luxation in toy and small breed of dogs.

Dismukes DI, Fox DB, Tomlinson JT, Essman SC, Cook JL. Determination of pelvic limb alignment in the large-breed dog: a cadaveric radiographic study in the frontal plane. Vet Surg 2008; 37: 674-82.

Table I

Angle	Free from MPL	Affected by MPL
Angle of inclination	130° ± 6.5°	130.1° ± 5.4°
aLPFA	115° ± 9°	112° ± 8.7°
aLDFA	95.3° ± 3.5°	100.2° ± 7°
mLPFA	105.1° ± 4.5°	107° ± 8°
mLDFA	103.1° ± 3.4°	104.3° ± 5.5°
Femoral anteversion	20.3° ± 4.8°	17.4° ± 7.5°
mCaPTA	65.4° ± 4°	74.8° ± 5.1°
mCrDTA	86.3° ± 1.5°	86.1° ± 2°
mMPTA	95.1° ± 3.2°	97.3° ± 6.6°
mMDTA	98.1° ± 4.4°	96.8° ± 3.6°
TPA	24.4° ± 3°	23.2° ± 5.6°

aLDFA, anatomic lateral distal femoral angle; mLDFA, mechanical lateral distal femoral angle; aLPFA, anatomic lateral proximal femoral angle; mLPFA, mechanical lateral proximal femoral angle; mCaPTA, mechanical caudal proximal femoral angle; mCrDTA, mechanical cranial distal tibial angle; mMPTA, mechanical medial proximal tibial angle; mMDTA, mechanical medial distal tibial angle.

COMPARISON OF THREE RADIOGRAPHIC METHODS FOR THE ASSESSMENT OF JOINT LAXITY IN CANINE HIP DYSPLASIA

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The ventrodorsal hip extended standard (VDS) view is a worldwide radiographic method used for the diagnosis of Canine Hip Dysplasia (CHD). Since the main problem of the VDS view is the minimization of the passive hip joint laxity, alternative methods were proposed.1,2 The purpose of this study was to compare 3 radiographic methods for assessing hip joint laxity.

From April 2012 to March 2013, dogs referred for diagnosis of CHD to the Veterinary Radiology Centre of Naples and to the Infirmary of the Veterinary Army Centre of Grosseto (Tuscany) were studied using 3 radiographic views: the VDS view, the ventrodorsal hip flexed and distracted view (VDD) and the ventrodorsal hip flexed and not-compressed view (VDF). Based on VDS, each joint was classified according to the FCI criteria (A, B, C, D, and E). On each joint, the Norberg Angle (NA) and the Distraction Index (DI) were measured. Comparison among the 3 views was made using the t-test for paired data; the correlation index was used to compare NA and DI measurements ($P < 0.05$).

Fifty-one dogs, of several breeds, 35 males and 16 females, 13.2 months of mean age (range: 4 - 34 months) were examined. According to the FCI criteria, 38 joints were classified as A, 25 as B, 17 as C, 14 as D and 8 as E. The mean NA (±St.Dev.) was 100.3° (±9.8°) in VDS, 93.4° (±13.6°) in VDD and 95.1° (±12.6°) in VDF. The mean DI (±St.Dev.) was 0.16 (±0.11) in VDS, 0.30 (±0.22) in VDD and 0.28 (±0.18) in VDF. VDD and VDF views had significant lower NA and higher DI values compared to those of VDS. No significant differences there were between VDD and VDF views. NA was significantly and negatively correlated to DI in all the three methods.

VDD and VDF views, both obtained with hip in a neutral position, had substantial similar results and showed to better highlight hip joint laxity than VDS view. Compared to VDD method, VDF does not require human operators or special devices for positioning the dog.

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EFFECTS OF BONE MARROW STROMAL CELLS (BMSCS) IN THE TREATMENT OF UNUNITED ANCONEAL PROCESS IN A YOUNG GERMAN SHEPHERD

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The purpose of this case report was to describe a possible alternative and innovative application of the cell therapy in the small animals orthopedic field.

In this case report we reported a case of a 7 months old, female German Shepherd dog affected by unilateral UAP, without obvious signs of articular incongruence.
At the clinical examination, the dog didn't show any signs of pain and important modification of the elbow joint were not evident.
The radiographic examination performed with the dog in general anesthesia revealed at the mediolateral projection the ununited anconeal process, without any signs of secondary DJD. The radiographic examination of the contralateral joint revealed the complete union of the ossification center of the anconeal process.
The computed tomography study of the left elbow revealed an evident and irregular fragmentation of the anconeal process of the olecranon, which was maintained in situ by fibrous tissue.
The owner was reluctant to an invasive surgical therapy, and thus a cellular therapy was proposed. At our days, BMSCs have been proven to be affective in the field of tissue engineering, indeed several studied reported the ability of these cells (alone or in conjunction with different substrates) to generate bone tissue.
The BMSCs were obtained from the bone marrow sampled at the level of the right ilium wing, by means of a Jamshidi biopsy needle, conneceted to a heparinized siringe (2500 IU/ 20 ml BM).The pellet obtained was suspended in 1 ml of Tissucoll.
The BMSCs that were obtained were injected, under fluoroscopy guidance, in the ossification center of the left anconeal process.

After treatment the dog didn't show any local or systemic adverse reactions. The radiographic and computed tomography follow ups at 13, 64 and 97 days after the treatment revealed the progressive fusion of the ossification center, which was completed at 97 days, without any signs of secondary DJD.

We can suppose that the results obtained in this single case may indicate a possible alternative and innovative application of the cell therapy in the small animals orthopedic field.

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ANALYSIS OF FACTORS INFLUENCING WOUND HEALING COMPLICATIONS FOLLOWING WIDE EXCISION OF FELINE INJECTION SITE SARCOMAS OF THE TRUNK

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Wide surgery is the mainstay of the multimodal treatment for injection site sarcomas (ISS) in cats.1 The deep infiltration of ISS in the surrounding tissues often force the resection of a wide area of cutis, subcutis, muscles and amputation of the underlying bony structures,1 making the subsequent reconstruction challenging. Only little data is available on post-surgical complications.2,3 The aim was to analyze the clinical, peri-operative, and histopathological factors influencing the development and timing of wound healing complications (WHC) in a homogenous sample population of cats undergoing wide excision of ISS located on the trunk.

Inclusion criteria were: newly-detected histologically confirmed ISS, tumor location on the trunk, no distant metastasis, no neo-adjuvant treatment, wide excision planned using contrast-enhanced computed tomographic (CT) examination and performed by the same surgeon, skin reconstruction by direct apposition of the skin edges or by local advancement flaps.
The prognostic effect of covariates (sex, age, weight, body condition score (BCS), site, clinical dimension (CD), computed tomographic dimension (CTD length and width), histotype, duration of surgery, excision pattern, reconstruction pattern, surgical margin status, local anesthesia) on total (Cox model), major (Fine and Gray model) and minor (Fine and Gray model) WHC was evaluated by univariate and bivariate analysis. The relationship between duration of surgery and clinical and imaging variables was evaluated.

Forty-nine cats were enrolled. No factors were associated to minor WHC onset. The main factor associated to the risk of total and major WHC was surgical time. Based on univariate analysis, pattern of reconstruction, CDT, CD, weight and BCS were significant prognostic factors for major WHC, but this was not confirmed in bivariate analysis. The duration of surgery was influenced by excision pattern and tumor CTD width.

Wide excision of ISS is unequivocally advocated to achieve a successful oncologic outcome taking the priority over any concern about WHC.1,2 However, a risk factor analysis on WHC is warranted because the development of postoperative morbidity affects the patients' outcome, implies a financial impact on the owner and may affect the timing of adjuvant oncologic therapy. An increased surgical time as the consequence of complex surgical procedures represented the best predictor for the development of WHC.

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EFFECTIVENESS OF TIBIAL PLATEAU LEVELING OSTEOTOMY (TPLO) AND INTRA-ARTICULAR INOCULATION OF AD-MSC AND HYALURONIC ACID IN THE TREATMENT OF OSTEOARTHRITIS SECONDARY TO RUPTURE OF THE CRANIAL CRUCIATE LIGAMENT IN DOGS

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To assess the evolution of OA in the stifle of dogs with CCL tear and treated with TPLO with and without intra-articular injection of allograft AD-MSC (adult mesenchymal stem cells derived from fat) and hyaluronic acid (HA).

Eleven dogs, 9 f, 2 m, mean age 4.4 y (1.5-9) and mean weight 35 kg (19-75), with a CCL tear underwent TPLO (T0) and were given injections of AD-MSC (2 mln/ml) and HA (20mg) (group1:8 dogs) or not (group 2:3 dogs). Control visits were performed at 15, 30 (T1), 60 (T2) days. t T0, T1 and T2 the degree of radiographic OA using the Kellgren-Lawrence scale was assessed. At T0 and T2 an SF sample, which has been tested for mucin, chemical-physical, cytological, and microbiological analysis, was taken.

No dogs had problems either during surgery or in the postoperative period. At 60 days after surgery (T2), 10 dogs showed full functional recovery of the treated limb.
In Group 1:
The degree (g) of radiographic OA was classified at T0: 0 g in 4 cases; 1 g in 1 case,3 g in 1 case, 4 g in 2 cases; at T1: 0 g in 4 cases, 1 g in 1 case, 3 g in 1 case, 4 g in 2 cases; at T2: 0 g in 4 cases; 2 g in 1 case, 3g in 1 case and 4 g in 2 cases.
At T0, the analysis of SF showed: chronicactive immune-mediated arthritis (1 case), mild chronic synovitis (5 cases), hemarthrosis (2 cases); at T2:all dogs showed an improvement in the quality and quantity of SF.
In Group 2,
At T0: 1 case had 0 g of OA and 2 cases grade 2; at T1: 0 g in 1 case and 2g in 2 cases; at T2: g 1 in 1 case, g 3 in 2 cases.
At T0, the analysis of SF showed: mild chronic synovitis (2 cases), chronicactive immune-mediatedarthritis (1 case); at T2: all dogs showed an improvement in the quality and quantity of SF.

These data support the literature regarding the usefulness of an early stabilization of the stifle with TPLO, following CCL rupture. It is known that no surgery can stop the progression of arthrosis. In this regard, some differences emerged between the group treated with AD-MSC and that not treated. All 8 group 1 dogs, had no progression of OA at T1 and at T2 only1 case had progression of OA. All 3 dogs of group 2, however, showed progression of OA at T2. It can, therefore, be assumed that the intra-articular injection of AD-MSC and HA contributes to reduce the progression of OA, particularly in those patients who are treated before the onset of joint damage. However, at 60 days after surgery, in all subjects an increase of GAG was found.
The finding that treatment with AD-MSC was always well-tolerated and that a delay in the onset or progression of OA is constantly verified. It is assumed that, in addition to their well-known regenerative features, AD-MSC , play an important role in the control of articular inflammation. A longer observation period and further case histories will allow more accurate evaluations. Our studies are currently aimed at assessing other elements of the SF, such as cytokines IL-1, IL-6 and TNF; MMP-3, TIMPs, KS-5D4, COMP.

References on request

A SURVEY OF FRACTURES IN STRAY DOGS OF NEAPOLITAN URBAN AREA

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To assess the prevalence and the distribution of bone fractures and the influence of age, breed, body size, and sex in the stray dogs of Neapolitan urban area, a retrospective study was made on the records of years 2011 and 2012 of the Regional Centre for Urban Veterinary Hygiene and the Veterinary Hospital Unit of the local public health NA1.

Age, breed, body size, and sex of fractured dogs were recorded. According to the age, dogs were classified in 5 groups: 1 = 1-6 months; 2 = 7-12 months; 3 = >1-5 years; 4 = 6-10 years; 5 = >10 years. According to body size, crossbreed dogs were divided in large (>25 kg), medium (≤25 >8 kg), and small (≤8 kg) size. Fracture features were assessed using radiographs. To evaluate age, breed, body size and sex influence, fractured dogs were compared to the overall population of dogs referred in the same period using the $\chi 2$ test ($P<0.05$).

On 3661 subjects referred, there were 342 fractured dogs (9.3%) for a total of 429 fractures (many subjects had multiple fractures). The sample was composed by 156 females and 186 males, with a prevalence of mixed breed (322 dogs; 75 large, 175 medium, and 72 small sized). Considering the fracture localization, femur (24%), radius-ulna (20.3%) and pelvis (18.9%) were the most common bones involved and appendicular skeleton was more represented than the axial one (respectively, 83.9% and 16.1%). The age distribution of fractured dogs was significantly different from the overall population with a higher proportion of dogs of group 1 and 2 ($P=0.005$) and a lower proportion of dogs of group 4 ($P=0.02$). Males were significantly overrepresented in the fractured dogs ($P = 0.1$). Considering breed and body size, there were no significant differences with overall population.

In our sample, the higher prevalence of fractured dogs, as well as the breed composition of the sample, directly reflects the typology of dogs considered. However, the distribution of bones involved and the higher prevalence of male and young subjects were consistent with previous reports.1,2 The knowledge of the prevalence and distribution of these lesions would be helpful in the clinical and resource management of the veterinary hospital facilities.

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ANAESTHETIC MANAGEMENT OF A SURGICAL TRACHEAL RUPTURE IN A CAT

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The aim of the study was to show the management of a tracheal rupture in a cat from the diagnosis to the surgical treatment.

A 2 years client-owned spayed female cat, was presented to the veterinary hospital for dyspnoea and anorexia. Lateral chest X-ray view was taken as first emergency diagnostic procedure. A tracheal lesion was identified above the base of the heart. A differential diagnosis was made amongst tracheal mass, tracheal malformation and tracheal traumatic rupture.

The cat was sedated with dexmedetomidine 2 mcg/kg and methadone 0.5 mg/kg intramuscularly and induced intravenously with a combination of ketamine and propofol (ketofol 1:1). Then 0.1 ml/kg of lidocaine 2% was used to desensitize the larynx. The cat did not revealed any respiratory problems after induction, also without endotracheal tube. Airway endoscopy was performed and it revealed a membranous web-like concentric stenosis without cartilage involvment, at this point the endoscopic probe could not proceed further. The cat was then intubated and maintained with isoflurane in oxygen. A computed tomography scan of the neck and thorax was performed, which revealed a tracheal rupture at the thoracic level. Lateral thoracotomy was planned for the day after. Next day the cat was induced intravenously with ketofol 2 mg/kg and it did not show any respiratory abnormality during induction. A 6 points (T2-T7) paravetrebral intercostal nerve block by electrolocation was performed using 0.3% ropivacaine. Anaesthesia was maintained with sevoflurane in 100 % oxygen and pressure mode mechanical ventilation was started with a preset peak airway pressure of 10 cm H2O, a respiratory rate of 25 breaths per minute and a PEEP of 4 cm H2O. Fentanyl 2-5 mcg/kg/h was administrated to improve analgesia.
Upon entry into the chest, there was an extensive tracheal mucosal bulla inflating during inspiration time. The endotracheal tube balloon was deflated to allow a better visualization of the mucosa. The mucosal bulla was then resected and a second sterile endotracheal tube was passed through the surgical incision. The breathing system was then connected to the new endotracheal tube. At the end of the surgical reconstruction, this tube was removed and the orotracheal tube was advanced over the tracheal lesion and mucosal suture performed. At the end of the surgery mechanical ventilation passed from controlled to assisted mode and the sevoflurane administration was suspended. The cat was positioned in sternal recumbency and 40 ml of air were aspirated from the right hemithorax. The recovery was uneventful.

The diagnosis of traumatic tracheal rupture of this clinical case was complicated. The intact tracheal mucosa was essential for the spontaneous ventilation of this patient. A good work team members in different disciplines (diagnostic, surgery and anaesthesia) was fundamental to plan the several steps to approach this pathology leading to an excellent outcome.

EVALUATION OF THE EFFICACY OF A NEW TRANSDERMIC FENTANYL SOLUTION ON THE PERIOPERATIVE PAIN MANAGEMENT FOR ORTHOPAEDIC SURGERY IN DOG: PRELIMINARY STUDY

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The aim of the study was to evaluate the analgesic effectiveness of a transdermic fentanyl solution for the intra-operative and postoperative pain control during orthopaedic surgery in dog

Fifteen dogs of various breeds and ages were enrolled in the study. Dogs admitted in the study were considered healthy on the basis of blood screening and clinical exam. Before the administration of 2.6 mg/kg of fentanyl transdermic solution, clinical parameters as heart rate (HR), respiratory rate (RR), blood pressure and temperature were recorded as well as pain and sedation scores. After fentanyl administration dogs were monitored each hour for 4 hours before the induction of anaesthesia and all the parameters recorded. Induction of the anaesthesia was performed with propofol and the maintenance with isoflurane in oxygen. During the procedure HR, RR, blood pressure, end-tidal CO2 (EtCO2), end-tidal isoflurane (EtIso) and temperature were recorded every five minutes. If an increase of the cardiovascular parameters more than 20% was recorded, fentanyl bolus at 2 mcg/kg was administered. At the end of the procedure time to reach palpebral reflex, extubation, head movements, sternal recumbency and standing were recorded. Pain check, with a Glasgow short form scale, was done every 2 hours for the first 8 hours after exubation and than every 4 hours for 48 hours. If pain score was more than 5 or 6 methadone 0.1 mg/kg IV was administered

In 2 cases a high sedation score was recorded before induction of anaesthesia. Propofol required for induction was 4-7 mg/kg. During surgery 2 dogs required rescue analgesia. Nine dogs required mechanical ventilation. HR and blood pressure were stable in 14 dogs. One dog required dopamine infusion for the maintenance of mean arterial pressure over 60 mm Hg. Mean time to extubation, head movement, sternal recumbency and standing were 17±12 min, 25±17 min, 46±28 min and 85±24 min, respectively. Two dogs required rescue analgesia in the postoperative period. A mild hypothermia was registered in the immediate postperative period in the majority of dogs. Urinary retention was seen in five dogs

This study revealed that the fentanyl transdermic solution, given 4 hours before the induction of anaesthesia, provided good analgesia both for the intraoperative and the postperative period. No adverse affects were recorded and an excellent cardiovascular stability was registered in all dogs in the intraoperative period. No evident sparing effect was recorded for the induction dosage of propofol while a sparing effect on isoflurane was identified for the maintenance of anaesthesia. Urinary output should be checked after the administration of fentanyl transdermic solution because of possible urinary retention.
Fentanyl transdermic solution at the dosage of 2.6 mg/kg was effective as sole agent for the perioperative pain management for orthopaedic surgery in dogs.

TEMPORARY PALATOPEXY FOR THE PREVENTION OF SHORT TERM COMPLICATIONS AFTER SURGICAL TREATMENT OF THE BRACHYCEPHALIC AIRWAY OBSTRUCTIVE SYNDROME (BAOS)

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The aim of the study is to assess a temporary palatopexy associated with the resection of the elongated soft palate and the correction of stenotic nares, to decrease the rate of short-term complications of the surgical treatment of the Brachycephalic airway obstruction syndrome(BAOS)

Eight English Bulldogs affected by BAOS,that underwent correction of stenotic nares and resection of the elongated soft palate,were included in this study. After premedication with Diazepam(0.2mg/kg iv) and Butorphanol(0.2mg/kg im), the anaesthesia was induced by Propofol(4mg/kg iv)and maintained with Isoflurane in 100% Oxygen. A temporary palatopexy was performed applying a horizontal "U" traction suture in PDS II® USP 2-0 between the middle point of the soft palate and the cranial third of the hard palate. The suture was tight enough to pull forward the soft palate and was removed 24h after surgery. Respiratory grading scores(1-3)as reported by Torrez(1),pulse-oximetry and spirometry were used to record the severity of the disease, before and after surgery. A follow-up time of 180 days was required for inclusion in the study. Differences between pre and post-surgical parameters were compared using the Kruskall Wallis test. Statistical significance was set at $p \leq 0.05$

The dogs weighed 23±5.7kg(mean±st.dev) and were 18.1±9.5 months old. Mean operating time was 33 minutes (range 30-45). No intraoperative complications occurred. Tracheostomy was not necessary in any case. Two dogs showed blood regurgitation, soon after extubation. Post-surgical oxymetry, heart rate, respiratory rate and respiratory scores differed significantly from preoperative values. Significant differences of the spirometric values were not recorded. All dogs showed improvement of respiratory signs at all times of the clinical follow-up.

Routine surgical treatment of BAOS includes correction of the stenotic nares and resection of the elongated soft palate, laryngeal saccules and excessive palatopharyngeal folds(1). Dyspnea is still the most common postoperative complication of these procedures. A combination of mucosal oedema, collapse of laryngeal structures and reduced pharyngeal muscle tone consequent to recovery from anesthesia are responsible for post-surgical respiratory distress(1). Postoperative dyspnea rate has been reported to reach 20.3%(1); this complication requires emergency tracheostomy in a percentage of cases varying from 5%(2) to 10.9%(3). Temporary palatopexy may reduce the incidence of short-term post-surgical complications through traction on the soft palate, which inhibits the laryngeal obstruction due to the collapse of oedematous laryngeal structures and reduces pharyngeal muscle tone. Thus temporary palatopexy combined with upper respiratory surgery seems to prevent the complication of postoperative dyspnea and improve the prognosis of dogs presented for BAOS

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IDENTIFICATION OF SAFE ANATOMICAL CORRIDORS ON THE LATERAL FACE OF THE VERTEBRAL BODIES OF THE THORACIC AND LUMBAR SPINE IN DOGS. PRELIMINARY ASSESSMENT OF SURGICAL STABILIZATION OF THE SPINE AS A RESULT OF TRAUMA.

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Objective: 1) Identifying repere (safety corridors) for implants (screws/pins) application; 2) evaluation of the ideal tilt angle, of reliability of choosen repere by ex vivo drilling test; 3) evaluation of differences between vertebrae over thoraco-lumbar spine.
Study Design: Computed tomography and ex-vivo prospective studies.

Materials and Methods: Phase I: CT images of spine of 20 dogs of different breeds were used to define lateral safe corridors in cross section plane. The angle allowing the highest amount of bone purchase with safe margins for bicortical implants was defined as the optimal corridor. Width and height of the vertebral body, optimum (α), maximum (β) and minimum (γ) safe angles were measured at the ideal insertion point. Phase II: 10 canine bodies belonging to different breeds were included. Standard surgical access and drilling, without introducing implants, were performed. The last 8 thoracic vertebrae and the whole lumbar spine were tested. Tests were performed by the same surgeon, over a 3 months period. All specimens were subject to CT to compare obtained tilt angles with ideal ones. To compare measurements performed on vertebrae of different shape and size, the absolute values were transformed in ratios. Continuous data were compared by a van der Waerden Analysis of Variance; post hoc differences were explored with Tukey's HSD.

Results: Insertion points were the vertebro-costal joint and the base of transverse process for thoracic and lumbar vertebrae, respectively. Optimum angle (α) was 90°. All three angles resulted significantly wider for almost all vertebrae compared to L7. The ratio between the safety margin and the vertebral height showed a significantly reduction in the last three lumbar vertebrae. Discussion: In vitro biomechanical studies on canine vertebrae demonstrated that an implant as near 90° to the longitudinal axis as possible increases the holding strength; such angle reduce risks of damaging of important anatomical structures1. Results of this study pointed out a progressive reduction, over a cranio-caudal direction, of the security margin; particularly, last two lumbar vertebrae showed a remarkable reduction of such margin, a characteristic that influence β and γ angles amplitude.

Conclusions: Lateral corridors used offer a good security margin and a good anchoring of synthesis means over the canine spine explored. Lateral approach allow easy screw orientation and a good visualization for bi-cortical anchoring.

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INTEGRATED IMAGING IN A MOUSE MODEL OF ACUTE COLITIS: PRELIMINARY RESULTS.

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Inflammatory Bowel Disease (IBD), which includes Crohn's and ulcerative colitis, can be a painful and debilitating condition. In addition to bowel symptoms, patients with IBD often experience extraintestinal complications, such as arthritis, kidney and liver disease, eye disorders and skin problems. (1). Actually nobody has found a technique to prove in vivo, on experimental model of colitis, the arthritis occurrence. Aim of our study was to find in mice the same occurrence than in humans with in vivo Imaging techniques (1-2).

Six mice (female), 6 weeks of age with a body weight of 18.5-22 g, were used for this study. 3 mice were studies 2 week after the induction of colitis, and 3 mice were used as control. To assess the presence of colitis, we used High Frequency Ultrasound (HFUS). To confirm the presence or the absence of joint damage inducted by colitis, mice were subjected to Positron Emission Tomography/Computed tomography (PET/CT) studies. HFUS were performed with the Vevo 770 (Visualsonics, CA) under general anesthesia. PET/CT studies were performed with a dedicated scanner (eXplore Vista, GE Helthcare) for small rodents. A dose of 250 µCi of [18]F-FDG was administered in a total bolus of 150 µl in the lateral tail vein. To facilitate the biodistribution of [18]F-FDG mice were stabulated at 26° C for 45 minutes. A static emission PET acquisition, lasting 30 minutes (1 bed positions), followed by a total body CT of about 10 minutes was performed. Total counts were calculated from the PET studies in right sacroiliac, and left sacroiliac joint in 3 mice 1 week after colitis induction and in 3 mice as control. A circular ROI including articular surface and joint space was chosen

Abdominal Ultrasound is effective in humans for identifying Crohn's disease or ulcerative colitis. In mice, we confirmed that HFUS is effective in determining the severity and damage associates to colitis. Like in humans, it is possible to identify colonic wall thickening and an increased intestinal mucosal plication and motion. Sometimes severe meteorism was also present. PET/CT assessed articular damage after colitis induction. In colitis mice there was an asimmetric [18]F-FDG uptake in joints considered in mice with colitis.

These results, encourages future studies to investigate the correlation between the disease and the intestinal articular humans.

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MAGNETIC RESONANCE IMAGING OF CEREBRAL CYSTIC LESION IN DOGS AND CATS

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Porencephalic cysts are congenital or acquired cerebrospinal fluid (CSF)-filled cavities within the cerebral hemispheres that usually communicate directly with the ventricular system and/or the subarachnoid space (1). The etiology of these malformations remains uncertain and several mechanisms have been proposed, as hypoxia-ischemia, toxicity, viral infection, vascular disease, mutation of specific genes, neuronal migration anomalies (2). Sometimes, these cystic lesions involve extensively the cerebral hemispheres (hydranencephaly). Magnetic Resonance Imaging (MRI) is the investigation of choice for the diagnosis of cystic lesions (3). Over the years different classifications of intracranial cystic lesions have been proposed in both human and veterinary medicine. These classifications are often incomplete, sometimes conflicting and based on different criteria. The aim of this study is to describe 15 unreported cases and review the literature and propose a new MRI-based classification.

Dogs and cats with a final diagnosis of either porencephaly or hydranencephaly were searched in this retrospective study (2007-2011). Inclusion in this study required an MRI study of the head. Data concerning breed, age, sex, medical history, clinical signs, MRI findings, and outcome were collected.

Eleven dogs and four cats fulfilled the inclusion criteria. Age at diagnosis ranged from 2,5 months to 8 years. In all cases where the neurological examination was available (n=13), the clinical signs correlated with the affected cerebral areas. Nine animals had seizures. MRI features of brain lesions were characterized by monolateral (9/15) or bilateral (6/15) cystic changes of the cerebral hemispheres of different shape and size. In 3 cases the MRI findings were not confined to the forebrain, being the cerebellum also involved,. Porencephalic lesions appeared as parenchymal defects connecting the ventricular system in 6 cases or both the ventricular system and the subarachnoid space in 8 cases. One of the animals had a history of postnatal brain trauma. Among the others, congenital problems, brain trauma or systemic infection were not reported. However, 9 of them presented skull remodeling similar to the one with the reported trauma.

MRI is helpful in the diagnosis of cystic lesions of the brain. Skull malformations can coexist and suggest an acquired rather than a congenital etiology in most cases. Cystic malformations can extend in posterior fossa, involving the hind brain.

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THE THORACIC ULTRASONOGRAPHY IN PATIENTS WITH ACUTE RESPIRATORY DISEASE

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Diagnosis of acute thoracic disease is a daily challenge for radiologists working in intensive-care units. It is often based on the results of chest radiography performed under technically unfavourable conditions. Radiographic quality is often inadequate, because patients are poorly collaborative, causing difficulties in the interpretation of the clinical presentation. The use of thoracic ultrasonography has recently been proposed in human medicine for the study of acute thoracic disease. It can be carried out rapidly with basic ultrasonographic equipment. The purpose of this paper is to present the experience in the use of thoracic ultrasonography in addition to radiology at the Teaching Hospital of the University of Pisa in patients with dyspnoea

From January 2012 to May 2013, 75 patients with acute respiratory disease were studied with ultrasound of the chest as a complement to radiographic examination. Radiographic examination was performed with an indirect digital system (Capsula-Fuji); the ultrasound equipment was a Xario (Toshiba) with a 7,5 MHz microconvex and 12 MHz linear multifrequency probe. Chest ultrasonography of both hemithoraces was performed with patients in sternal or lateral recumbency, according to the clinical condition; longitudinal and transverse scans at intercostal spaces were taken, sliding the probe dorso-ventrally and progressing craniocaudal. We evaluated the sonographic patterns described by Lichtenstein, based on pleural movements and on artifacts generated by the passage of ultrasounds in the lung parenchyma

Based on the ultrasonographic findings patients were divided into 5 categories: pneumothorax (8), pleural effusion (32), diaphragmatic hernia (7), alveolar consolidation (13, of which 9 aspiration pneumonia and 4 pulmonary contusions), alveolar interstitial syndrome (15, of which 8 cardiogenic pulmonary edema and 7 non cardiogenic). All radiographically detected conditions were also highlighted by ultrasound; only ultrasonography allowed the identification of small fluid pockets and/or pneumothorax in complex diseases, and to differentiate a non-cardiogenic pulmonary edema, from a cardiogenic one

The prompt detection of the presence of pleural movements ("lung sliding") and the presence of "comet-tail" artifacts (B lines), allowed to identify disease of both of the pleural space and of the lung parenchyma. Ultrasonography yields a quicker diagnosis than radiography, with less discomfort of the patient; an interstitial-alveolar pattern can be identifed early, when the proportion of extravascular water is not detected by radiography. It is also relevant in the monitoring of patients, as a response to therapy, for example in cases of cardiogenic pulmonary edema, or as reduction of the pleural pathology, in the case of chest drainage.

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MAGNETIC RESONANCE IMAGING FEATURES OF DIGASTRIC MUSCLE ROSTRAL AND CAUDAL BELLIES IN DOGS WITH EITHER TRIGEMINAL OR FACIAL NERVES DYSFUNCTION

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The digastric muscle (DM) belongs to the group of masticatory muscles (MM). In dogs it appears as a single-bellied muscle; however, a tendinous intersection links a rostral belly and a caudal belly, innervated by the trigeminal nerve and the facial nerve, respectively (1,2,3). The DM cannot be examined directly but it is easily identified by means of magnetic resonance imaging (MRI)(4).
The aim of this study is to investigate whether 1) denervation atrophy of DM rostral or caudal parts develops as a consequence of trigeminal nerve dysfunction (TND) or facial nerve dysfunction (FND), respectively, and 2) correlations between the clinical and imaging features and age, body weight, cranial conformation, gender and course of the clinical signs do exist.

Dogs with neurological signs of either facial or trigeminal nerve unilateral dysfunction were searched. Inclusion in this study required documentation of a complete neurological examination on presentation and an MRI series of the head being available for reassessment. Furthermore, a control group was created searching for patients with a negative MRI study of the head. In each MRI, the border of the caudal and cranial bellies was outlined manually with a digital cursor, and its cross-sectional area was computed by the "area" function of the OsiriX software.

Thirty-three dogs with FND and 15 dogs with TND were included in the study. No correlations with gender, body weight and side of the lesion were identified. In the group of TND most of the dogs were mesocephalic and old (> 7 years); all had a chronic course of the clinical signs (> 15 days). Most of them were diagnosed with a peripheral nerve sheath tumor. The majority of dogs in the FND group were old with a homogeneous distribution regarding skull conformation and the course of clinical signs. On MR images, in both the FNP group and TND group, the DM caudal and cranial belly cross-sectional areas, respectively, were significantly ($p<0.001$) lower on the affected side than those on the controlateral side. In the control group, differences between cross-sectional areas of right and left DM bellies were not significant. No correlation was found between the severity of muscle atrophy and the course of the clinical signs.

MRI is helpful to demonstrate the denervation atrophy of the DM cranial or caudal belly in clinically diagnosed TND and FND, respectively.

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NON MINERALIZED TENDINOPATHY IN DOGS

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Supraspinatus tendinopathy (ST) has been reported in dogs but only 10 cases of nonmineralized ST has been described. Although the cause of ST is unclear, suggested contributing factors include aging, overuse, trauma and hypoxia secondary to hypovascularity of the supraspinatus tendon.
We retrospectively evaluated clinical signs, imaging findings, surgical treatment and outcomes in dogs with nonmineralized ST.

Medical records (2009–2010) of dogs diagnosed with nonmineralized supraspinatus tendinopathy that had surgical treatment were reviewed. Patients with complete orthopaedic examination, shoulder pain, radiographic examination of the shoulder, MRI and surgical treatment were included in the study. After MRI confirmed the suspected non mineralized supraspinatus tendinopathy, surgery was performed. After arthroscopy, a craniomedial approach to the supra- spinatus tendon and intertubercular groove was performed. The supraspinatus tendon was thoroughly digitally palpated and inspected for color and consistency, then the most affected part was resected and full thickness longitudinal incisions were performed along the tendon of insertion of the supraspinatus muscle.

Six dogs met the inclusion criteria. Median age on admission was 3 years. Breeds were German shepered (1), American Staffordshire terrier (1) , Russian terrier (1), boxer (2), Caucasian shepherd (1). All dogs had unilateral chronic thoracic limb lameness. Median (95% CI) duration of clinical signs was 3 months (range, 1-6 months). Pain on palpation of the affected shoulder was the most consistent finding during orthopedic examination. In all patient was perfomed arthoscopy and surgical treatment. After surgical treatment, recovery was judged excellent in 5 patients and poor in one case. Two patient were complicated by seroma formation.

Most dogs in our study were very active dogs performing frequent high impact activities. Indirect and repetitive trauma to the shoulder region and overuse exercise have been proposed as causes of mineralization of the ST.The most consistent findings on MRI were enlargement and increased signal in the area of insertion of the supraspinatus tendon over the greater tubercle of the humerus on T2-W sequences. We performed full thickness, longitudinal incisions in the supraspinatus tendon. This technique could decrease intratendinous pressure, increase vascularization of the critical zone, and induce healing, improving or reversing degeneration. Resection of the most medial aspect of the tendon, and in some cases the transection of the transverse humeral ligament release impingement of the biceps tendon.

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RADIOLOGIC ANATOMIC VARIATION OF THE CAUDAL CERVICAL VERTEBRAE IN HORSES: PRELIMINARY RESULTS.

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The purpose of this study was to describe the variation of the radiologic appearance of the caudal cervical vertebrae in horses of different breeds, discipline and gender with cervical pathology and control horses.

The clinical record of horses with lateral radiographic projections of the cervical spine performed between January 2006 and May 2013 were reviewed. There were a total of 257 horses of different breeds;106 were females,95 genders and 56 males. Age ranged from 6 months to 34 years. For statistical analysis of the clinical significance of radiologic findings, horses were grouped in group N=normal (n=222) and group A=abnormal (n=35) based upon the presence of cervical pathology. The presence of the spinous process (SP) of the seventh cervical vertebra (C7) and of the first thoracic vertebra (T1), of a small centre of ossification at the caudal limit of the ventral process of the sixth cervical vertebra (C6) and the transposition of the trifid transverse process (TrTP) of C6 onto the ventral surface of C7 (partial or complete) were recorded. Radiographs were reassessed and a classification system was devised to describe the shape of the SP of C7(triangular,rounded and spur-like), of the SP of T1(high and pronounced or short and squat). X2-test were used to test for associations between radiologic findings and gender, breed and discipline, to test for association between different radiologic findings and to test for associations between radiologic finding and horse groups. The statistical significance was set at $P< 0.05$.

The 46,3% of horses had a SP on C7,of these 61,4% had a triangular shape, 29,4% had a rounded shape and 9,2% had a spur-like shape. Only the 2,4% of horses had a centre of ossification at the caudal limit of the ventral process of C6. The SP of T1 was high and pronounced in the 75,5% of horses and short and squat in the 24,5%. TrTP was identified in 37 horses and it was complete in 13 and partial in 24.Variation of radiologic appearance of the caudal cervical vertebrae was not associated with breed, gender and discipline; a tendency was identified for TrTP of C6 onto C7 and sex.
A significant association between the presence/absence of the SP of C7 and the shape of T1 was identified and the shape of T1 was associated with the presence/absence of TrTP of C6 on C7. There was an association between the absence of the SP of C7 and the short and squat shape of the SP of T1 and the group A.

Most radiologic anatomic variations of the caudal cervical vertebrae were not associated with breed, gender and attitude; just a tendency was identified for TrTP of C6 onto C7 to have higher frequency in females. The absence of the SP of C7 and high and pronounced shape of the SP of T1 was the most common variation. The absence of the SP of C7 and a short and squat shape of SP of T1 were associated and both had higher frequency in horses with cervical pathology.

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B MODE AND PD EVALUATION IN THE HORSE'S TENDINOPATHY AND DESMITIS

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The lesions of the suspensory ligament and digital flexor tendons injuries are commonly in sport horses.The aim of this study was to investigate in horses with TP and desmitis the existence of relationship between tendon and ligament neovascularization measured by PD and clinical severity parameters of TP and desmitis.

25 tendons were examined. For the PD examination the limb was flexed to restrict the movement and the structures were examined in longitudinal and transverse planes .It was scored using Filippucci-Grassi semi - quantitative scoring system.
In the horses Autologous BMSC implantation into the core lesion was carried out. For the rehabilitation the horses have done a program of training , clinical , B mode and PD ultrasound control at 30-60-90 days.

In 23 cases the symptomatology and the US and PD images have improved , only 2 horses were lame and without clinical ,US and PD improvements. The results of this study suggest a correlation between PD activity , echo score e FAS in B mode and clinical symptoms .In fact,it was confirmed that in the normal tendon structure isn't possible to verify the presence of PD signal within the tendon tissue ,but only in the inter tendinous space.In contrast, in the tendons that have a injury in both acute and chronic symptoms, neovascularization that characterizes the inflammatory process is put in evidence with the PD through the increase in signal that is distributed not only in inter-tendinous space ,but above all inside of the injured tendon.

Our study has just revealed a higher specificity than US image PD and morphological changes as shown in B mode are often related to the presence of vascularization visible with PD.One of the first goals is standardization of the technique, as, for evaluations of tendon injuries of the horses, we must find how to place the animal to minimize the movements that interfere with visualization of the vessels with the PD.In conclusion we can say that the PD is a great diagnostic aid in the evaluation of equine tendon injuries, and it has also the advantage of being easy to use for those who are familiar with methodologies ultrasound.In this study, the survey PD has been shown to provide a wide range of comprehensive information concerning the monitoring of recovery and prognosis.

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ULTRASONOGRAPHIC FINDINGS IN HORSES WITH SEPTIC ARTHRITIS/TENOSYNOVITIS: PRELIMINARY RESULTS

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The purpose of this study was to describe the ultrasonographic characteristics of equine septic arthritis/tenosynovitis consequence of traumatic and iatrogenic causes.

Thirty-eight horses with confirmed septic arthritis/tenosynovitis that underwent an in-depth ultrasonographic examination between January 2005 and March 2013 were included in this study. A total of 43 joints/sheaths were evaluated. Diagnosis of septic arthritis/tenosynovitis was based on historical and clinical findings as well as the results of white cells blood count of the synovial fluid (>30.000 cells/µL), in horses where synovial fluid was available, and the positivity of synovial colture. Ultrasonographic findings evaluated were degree of joint/sheath distension (absent, mild, moderate/severe), degree of synovial membrane proliferation (mild, moderate/severe), appearance of synovial fluid (anechoic, echoic), presence of synovial spots and fibrin-like material. Ultrasonographic findings were tested for dependence by cause of sepsi (wound=29 cases, others=16), by time between admission and beginning of clinical signs (<24h=16 cases, >24h= 27), and by white cells blood count of synovial fluid (< 30.000 cells/µL, > 30.000 cells/µL) using chi-squared test and calculation of coefficient b of regression for 2x2 tables. Significant was set at P<0.05.

Age ranged from 4 months to 19 years. Breed was 17 Warmblood, 13 Thoroughbred, 3 Standardbreds, 2 Quarter Horse, 1 Arab and 2 of unknown breed. Affected synovial structures included 20 digital sheaths, 5 tarsocrural joints, 4 antebrachiocarpal joints, 4 medial femorotibial and femoropatellar joints, 5 metacarpo/metatarsophalangeal joints, 2 middle carpal joints, 2 distal interphalangeal joints e 1 lateral femorotibial joint. Ultrasonographically, the degree of joint/sheath effusion was considered absent in 1 case, mild in 7 and moderate/severe in 35 cases; the synovial membrane was considered with mild hypertrophy in 13 cases and with moderate/severe hypertrophy in 29. The synovial fluid was anechoic in 19 cases and echoich in 23. Hyperechoic spots were identified in 14 structures e fibrin-like material in 27. Statistical analysis identified a correlation of the degree of synovial effusion, the proliferation of the synovial membrane and the appearance of the synovial fluid by the time between admission and the beginning of clinical signs, and of the presence of fibrin-like material by the cause of the sepsis.

The ultrasonographic examination is an important diagnostic aid in cases of synovial sepsis. The ultrasonographic findings of septic arthritis/tenosynovitis can be various and attention should be put on evaluation of degree of effusion, synovial membrane proliferation and echogenicity of synovial fluid because they are influenced by time between admission and the beginning of clinical signs.

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SEPTIC TENOSYNOVITIS CAUSED BY PORCUPINE QUILLS : DIAGNOSIS, TREATMENT AND LONG TERM OUTCOME IN 7 HORSES.

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The study describes the diagnostic trial, treatment and outcome of horses referred for a suspected infected digital tendon sheath (DFTS) due to a puncture wound caused by a porcupine quill.

Seven horses were referred to our Veterinary Teaching Hospital (VTH) for a wound caused by porcupine quills and suspected penetration on the DFTS, with a total of 8 limbs involved. The diagnosis of sepsis of the DFTS was made with a clinical, radiographic and ultrasonographic examination followed by synoviocentesis; all affected DFTS underwent tenoscopic debridement and flushing under general anesthesia.

Horses aged from 3 months to 3 years, with wide breed distribution; all were from moderately to severely lame at presentation. Porcupine quills were present in one horse, whereas the owner or the referring vet removed them in the other patients. All horses had abnormalities on ultrasound examination that ranged from severe distention of the DFTS with anechoic\echoich fluid to full thickness injuries of tendons or ligaments within the sheath. The white blood cell count was high in all the samples taken and bacteria were isolated in only 2 cases. Tenoscopy confirmed the lesions found with the ultrasound in all the patients but it revealed occult lesions in 2 DFTS. All horses received systemic and local antimicrobial therapy. All patients were discharged from the hospital when they were sound at walk; the length of hospitalization ranged from 4 to 23 days. Follow up information were available for all the patients from 8 months to 4 years: 1 horses did not reach the expectations of the owners and is performing at lower level, however the others were able to return to their intended use. The DFTS appearance was considered acceptable for 1 horse and good the others.

Porcupines are nocturnal herbivores diffused in a wide part of the world, their body is covered by quills and they are released on contact; accidental encounter with horses can cause wounds on the distal limb. A case of DFTS infection due to a porcupine quill in a horse is already described in literature; however in the present study a larger case series is considered and the outcome can be compared to other data present in literature. Nevertheless we found severe damage of different DFTS structures the outcome can be considered better in this study compared to a recent one where only approximately 50% of horses treated for septic DFTS returned to their previous level of performance.

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PREVALENCES OF DEVELOPMENTAL ORTHOPEDIC DISEASE (DOD) IN YOUNG HORSES BRED IN BELGIUM AND IN SARDINIA. RADIOGRAPHIC SCREENING.

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To determine prevalences of DOD and joints more affected, by the detection of radiographic signs, in two groups of young horses, bred in different breeding conditions: Belgium and Sardinia.

Two groups of horses were considered. The belgian group, 676 stallions, aged 3.27 ± 2.04 years, weighting 530 ± 41.09 kg, presented for admission to the "Royal Belgian Sports Horse Society". The sardinian group, 24 horses, aged 3.75 ± 1.35 years, weighting 420 ± 40.5 Kg, presented for pre purchase examinations. In the belgian group, official report of the lectures was established by agreement between two ECVDI radiologists. In the sardinian group, final report was established by agreement between the veterinary practitioner and an ECVDI radiologist. Radiographic images were recognised according to the classification described by Denoix and were assigned a Radiographic Score (RS) of severity, where Abnormal Radiographic Images (ARI) had RS 2, 4 or 8, while Suspected Radiographic Images (SRI), had always RS 1 (1).
By Z-test ($P<0.05$), for each joint in both groups of horses, prevalence of ARI and SRI, as well as prevalence of the most detected DOD and RS were determined.

In the front foot, ARI plus SRI were found in 77.7% of the belgian group with RS 0.6 and in 95.8% of the sardinian group with RS 1.2. Synovial distension of the dorsal recess of the distal interphalangeal joint was found in 45% of the belgian group and in the 66% of the sardinian group.
In the fetlock, ARI plus SRI were detected in 51.4% of belgian group with RS 1.3 and in 83% of sardinian group with RS 2.4. The most represented lesions were the irregularity of the proximal border of the sagittal ridge of the McIII and MtIII in belgian group (16.1%) and remodeling of the proximal border of proximal phalanx in sardinian group (37.5%).
In the hock, both in belgian and in sardinian group, only SRI were found, with prevalence of 22.3 in the first and 8.3 in the second group, always with RS 1. Bony spur at the dorsoproximal margin of the MtIII was detected in 8% of the belgian group while it was absent in the sardinian group.
At the stifle, only ARI were found in the belgian group, with prevalence of 8.4% and RS 5.6, while none ARI but only SRI were found in the sardinian group (12.5%) with RS 1.7. Osteochondrosis at the femoral trochlear ridges without fragments was the lesion most detected in the belgian group (5%), while flattening at the femoral trochlear ridges was detected in the sardinian group (12.5 %).

Higher prevalence of DOD at hock and stifle in the belgian group agreeds with literature (2,3) and it correlates with the greater weight of the horses of the belgian group. On the contrary, sardinian horses are less affected by degenerative lesions, but more affected by lesions of traumatic origin at the front foot.

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CERVICAL ARTHROPATHY IN A HAFLINGER MARE

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To describe a peculiar case of cervical osteoarthrosis in a Haflinger.

A 7-year-old, Haflinger mare was presented to the DVCS with a 6 months history of nasal discharge of ingesta and fluids, poor performances, inspiratory respiratory noise and cough during exercise, weakness and sweating at rest.
The clinical examination revealed rectal body temperature of 38.2°C, heart rate 48 bpm, respiratory rate of 30 acts/min with left nasal reflux of ingesta and neck sweating.
Endoscopy of the upper airways revealed pharyngeal collapse, left recurrent laryngeal hemiplegia (grade IV) with left slap test failure and food contamination; no alteration of guttural pouches was evident. The neurological examination showed normal mentation and no gait abnormalities. Abnormalities of cranial nerves such as the gag reflex, skin sensation in the cervical region, cervicofacial and cervical panniculus were detected on the lesft side. Neck conformation, shape and posture at rest were normal but a slight swelling at left proximal cervical region was evident. Left lateral neck flexibility was reduced. Left facial asymmetry with a right deviation of the upper lip, left eyelid ptosis and protrusion of the third eyelid occurred. Intermittent sweating around the base of the ears, left facial region and neck was noted in the same area of swelling.
Pupil size and pupillary light reflex, eye position and symmetry were normal.
These neurologic findings suggested a left caudal brainstem and cranial cervical spinal cord neuroanatomic localization (cranial cervical ganglia) and partial Horner's syndrome.
Cranial and cervical radiographs were performed under sedation in standing position (1). Lateral-lateral (exposures from left to right and right to left) and dorsal-ventral images were obtained and showed left occipito-atlanto joint remodeling and sclerosis, with bone remodeling of the dorsal surface of the dens of the axis.
Triamcinolone (20mg) and betametasone (2mg) were inoculated transendoscopically on the floor of the medial compartment of the left guttural pouch in proximity of the IX-X-XII cranial nerves (sympathetic trunk).

On the basis of clinical/neurological exam and diagnostic imaging a tentative diagnosis of occipito-atlanto osteoarthrosis can be established.
The treatment allowed complete remission of clinical signs for 7 days.
Ultrasonography, arthroscopy and eventually CT would be necessary to confirm the diagnosis. Cost limitations did not allow to proceed with the diagnostic process.

To our knowledge this is the first report describing occipito-atlanto osteoarthrosis associated with partial Horner's syndrome in a horse. The lack of specific literature highlights the low incidence of the pathology (2), and the limitations in the diagnostic process and therapeutic approach.

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USE OF INTRAVENOUS INJECTION OF AGITATED SALINE FOR THE ULTRASONOGRAPHIC DIAGNOSIS OF TOTAL JUGULAR VEIN OCCLUSION

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Jugular vein is the most common site for venous thrombosis in the horse, usually secondary to intravenous injections or catheterization, although thrombosis and thrombophlebitis can occur in the absence of pre-existing vascular trauma. The thrombus may occlude the lumen of the vein, resulting in perivenous and facial edema and dilatation of facial veins on the affected side, proximal to the thrombus. Ultrasonographic diagnosis is useful in detecting type and progression of thrombosis, and its characterization as well as aiding in surgical planning. Nevertheless bidimensional and Doppler evaluation is often complicated and very operator dependant. The aim of this work is to test agitated-saline contrast ultrasound for evaluating permeability and evolution in a case of jugular thrombosis.

A 5 y old gelding brown was presented with dilatation of right side facial veins. The right jugular vein could be palpated as thick, solid structures in the jugular groove. No manual congestion could be achieved. A wide range linear 4-11 MHz vascular transducer was used to scan both jugular veins. Bidimensional ultrasonography of the affected vein showed a homogeneous hypoecoic thrombus occluding a narrowed right jugular vein for about 30 cm of length in the proximal and mid portion of the neck. Afterwards, 40 ml of 0,9% NaCl solution were agitated back and forth between two syringes connected at right angles by a three-way stopcock. A 20G intravenous catheter attached to the three way via a flexible extension tube was placed in the right congestive facial vein.
During injection of agitated saline the transducer was positioned in two acoustic windows: the first on right jugular vein, just caudal to the thrombus, the second on left jugular vein, immediately proximal to the thoracic inlet. A 30 s cineloop was then recorded in each acoustic window after the injection of each single bolus.

No microbubbles were retrieved in the right jugular vein, beyond the thrombus, after injection of the first bolus of agitated saline. Microbubbles were visualized in the left jugular vein as a small, intense echo signal within the vein lumen after injection of the second bolus, demonstrating the presence of collateral vein circulation.

The absence of microbubbles beyond the jugular thrombus makes injection of agitated saline in facial veins a candidate as an adjuvant technique for the diagnosis of total jugular vein occlusion. Although further studies including more cases are needed, this easy technique could serve as a good tool in collateral circulation detections and in decision making in this pathology.

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MAGNETIC RESONANCE IMAGING FEATURES OF ETHMOIDAL EMATHOMA IN THE HORSE

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Ethmoidal Hematoma is a non-neoplastic pathology of the paranasal sinuses, with unknown eaetiology, that represents 10% of the diseases of this region. Clinical signs are rhinorrhagy, nasal discharge and respiratory noises, while endoscopy and biopsies show masses of blood and fibrous tissue encapsulated within normal respiratory epithelium.
Magnetic resonance (MR) imaging provides excellent anatomical detail of equine head and an accurate support for the diagnosis of the upper respiratory diseases in horses.

Two horses, females, of different ages and breed were imaged. They presented dispnea, respiratory noise and nasal hemorrhagic discharge. In both cases diagnosis of Ethmoidal Hematoma was made by endoscopy and confirmed by histologic examination. One horse had recurrence after surgery and has been examined twice.
A low field open magnet (Paramed, 0.23 Tesla) was used. The horses were positioned in left lateral recumbency under general anesthesia. The coil was applied on the frontal region and the eye of the horse was placed in the isocenter of the magnet. Sequences in the standard protocol were T2W fast spin echo, T1W spin echo in the transverse, dorsal and sagittal planes. Mean scan time to complete the examination was 42 min (range 28-55 min).

Frontal and maxillary sinuses, meatus nasi, dorsal and ventral choncae resulted deformed and not easily recognizable. Lesions appeared expanded in the controlateral nasal cavity. The signal was hyperintense by a secondary sinusitis for airway obstruction and fluid accumulation. The most useful sequences was T1W in dorsal and transverse planes.

Commonly, the features of Ethmoid Hematomas includes masses originating within the ethmoid turbinates with encapsulated contents clearly visible and distinguishable. The particular appearance of our cases can be explained by the clinical status, by the presence of fluid and/or blood and also for size, severity and chronicity of the lesions. MR is a useful tool in Ethmoidal Hematoma diagnosing, an important support to program surgical planning and to made a prognosis considering the involvement of the tissues and the real extend and nature of the lesions.

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POSTOPERATIVE FEVER IN THE HORSE: RELEVANCE OF PIROPLASMOSIS AMONG THE DIFFERENTIAL DIAGNOSES

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Infections are common complications in the post-operative period of horses which develop pyrexia. In Italy is common to have positive results after serologic exams for piroplasmosis (Pir) in horses with fever1. The aim of the study was to determine the relationship between type of surgery, piroplamosis, infection and fever.

Three hundred and sixteen horses underwent surgery between January 2010 and March 2013 were included. Selected data were: gender, age breed, pyrexia (temperature >38.3°C) onset of temperature (less or more than 48h after surgery), duration and peak of temperature, type of surgery [elective orthopaedic (EO), elective soft tissue (EST), emergency orthopaedic (EmO), emergency soft tissue (EmST), laparotomy (LT) and laparoscopy (LS)], site of infection, serologic exams for Pir with indirect immunofluorescence Level of pyrexia was classified in 5 groups: no fever (A), 38.3-38.8°C (B), 38.9-39.4°C (C), 39.5-39.9°C (D), >40°C (E)2. Chi-squared or Fischer test were used, when appropriate, to test for association between type of surgery and pyrexia group and presence of Pir, between presence of Pir and pyrexia group, duration of pyrexia and, the onset of temperature. Parametric and continuous data were test for normality with Shapiro-Wilk test and for homogeneity of variance with Levene test. Peak of fewer were tested for difference between horses with and without Pir, for each type of surgery group. Level of significance was set at $P<0.05$.

The EO included 100 patients, in the EST there were 75 horses, in the EmO there were 16 horses, EmST included 11 horses, LT included 90 horses, the LS 24 horses. Twenty-one horses were positive for Pir. Forty/316 horses developed infection and 10/316 had both Pir and infection. There was positive association between LT and fever groups C, D and E, but a negative association with group A. A positive association was found also between LS and group D, between EO and group A and a negative association with groups C, D, E. Pir was positively associated with LS and negatively with EO. In LT there was a positive association between Pir and group E, between absence of Pir and group B and a negative association with group E. Similar result was obtained in LS but the positive association was between absence of Pir and group B. In EST, the group D was positively associated with Pir and negatively with absence of Pir. The peak temperature was higher for horses with Pir (mean 40.5°C) than without (mean 38.8°C) and, than with infection (mean 39.3). The were no differences about time of onset of temperature in horses with and without Pir, duration was longer in horses with Pir.

Results are important for diagnosis in horses with pyrexia in the postoperative period, especially in soft tissue surgery. The odds ratio for Pir was a higher in LS, LT and EST, in horse with Pir the temperature was higher, duration was longer.

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EVALUATION OF A HOCK RADIOGRAPH READING SYSTEM FOR SCREENING PURPOSES IN YOUNG HORSES

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To evaluate the validity of a pre-established Radiographic Reading System (RRS) for detection of radiographic signs of Developmental Orthopaedic Diseases (DOD) in the equine hock.

Out of 350 stallions screened for admission at the Belgian Studbook, 135 hocks (4 standard views) were selected so that the sample contained the most common DOD lesions. Of the selected hocks, 22 were DOD free and 113 had radiographic changes compatible with DOD. Radiographs were read in blind by 8 readers (one of them defined as the expert as routinely involved in radiographic screening plans) using a dedicated RRS in the form of a grid (Table 1). An official report by the official Studbook commission was available for each hock. Data were analysed statistically using a Z-test ($P<0,05$) in order to compare:
- the official report versus the reading of the 4 views by the 8 readers;
- the reading of the LM view alone and of the 4 views by the expert versus the reading of the LM view and of the 4 views by the other 7 readers.
Agreement between readers was determined by the percentage of total agreement (Po) and by the multirater free-marginal kappa (kfree).

Comparison between the official report and the reading of the 8 readers in the 4 views showed significant differences only for medial malleolus osteochondrosis and for degenerative changes of the dorsal tarsometatarsal joint margins.
Comparison between the reading of the LM view between the expert and the other 7 readers was significantly different only for detection of subchondral bone cyst-like lesions and for degenerative changes in the dorsal margins of distal tarsal joints. The expert detected cyst-like lesions more frequently, whereas less experienced readers detected more distal tarsal joints changes. Difference in reporting degenerative distal tarsal joints changes was also significant when comparing the readings of the 4 views.
Agreement between readers in classifying the hock as osteochondrosis positive or negative resulted optimal for the reading in the LM view alone (Po=0.93; Kfree=0.86), substantial for the reading in the 4 views (Po=0.82; Kfree=0.65).

Minor differences were seen between the official report and the reading by the 8 readers suggesting that a RRS helps less experienced readers in identification of radiographic changes. Differences in detection of cyst-like lesions shows that identification of low contrast abnormalities in bone requires a higher degree of experience if compared with identification of abnormalities altering profile and shape of the bone. Less experienced readers tend to overestimate degenerative changes in distal tarsal joints. The optimal agreement between readers strongly supports the use of a RRS in screening protocol of sires (3).

1) OLIVIER A. et al., J. S. Afr. Vet. Ass. 68, 125-129, 1997; 2) LABENS R. et al., Vet. Rad., Ultr., 48(3): 204-11, 2007; 3) GRONDAHL A. et al., J. Am. Vet. Med. Ass. 203:101–104, 1993.

JOINT	RADIOGRAPHYC SIGN
Tibio-tarsal joint	Flattening of talar ridge (specify if medial or lateral)
	Notch in the talar ridge (specify if medial or lateral)
	OC of the talar ridge with changes in bony profile and opacity (specify if medial or lateral)
	OC of the talar ridge with fragmentation (specify if medial or lateral)
	Fragment at the distal extremity of the lateral talar ridge
	a: Flattening **b**: Notch or irregular bone margin of tibial cochlear ridge (OC without fragment)
	OC of the tibial cochlear ridge with fragment
	Prominent proximal tubercle of the talus
	OC of the proximal tubercle of the talus (fragment)
	OC of the maleolus (specify if medial or lateral)
	Subchondral bone cyst in the distal tibia
	Subchondral bone cyst in the talus
	Subchondral bone cyst in the calcaneus
	Dorsal modelling / osteophytes distal tibia
Proximal intertarsal joint	Dorsal modelling / osteophytes
	Loss of visibility of the joint space
	Narrowed joint space
	Loss of corticomedullary definition
	Subchondral bone sclerosis
	Subchondral bone lysis
	Subchondral bone cyst
Distal intertarsal joint	Dorsal modelling / osteophytes
	Changes in opacity of the joint margins
	Loss of visibility of the joint space
	Narrowed joint space
	Loss of corticomedullary definition
	Subchondral bone sclerosis
	Subchondral bone lysis
	Subchondral bone cyst
Tarso-metatarsal joint	Bony spur on Mt III
	Dorsal modelling / osteophytes
	Changes in opacity of the joint margin or of the bony spur
	Loss of visibility of the joint space
	Narrowed joint space
	Loss of corticomedullary definition
	Subchondral bone sclerosis
	Subchondral bone lysis
	Subchondral bone cyst
Third Interosseus Muscle insertion	Thickening of the plantar cortex
	Bony proliferation on the plantar cortex
	Avulsion fragment at the origin

Table1: Grid for the radiographic reading system, containing most common radiographic signs and radiographic lesions encountered in the equine hock.

STANDING THORACOSCOPIC DIAPHRAGMATIC HERNIA REPAIR IN A HORSE BY A NEW DUAL FACING MESH

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This report describes a successful thoracoscopic repair of a diaphragmatic hernia (DH) using a dual-facing mesh made of polyester and absorbable hydrophilic film in a standing horse.

A 6-yo gelding was referred for severe signs of colic, which required surgery under general anesthesia. At abdominal inspection the diagnosis of small intestine strangulation in a diaphragmatic tear was made. The tear was dorsal to the spleen in the tendineous part of the diaphragm. The herniated viscera were not manually reducible and a further enlargement of the defect was necessary. An enterectomy of 6 m of jejunum was performed. Because of the dorsal location of the tear, herniorrhaphy was technically not feasible. Five days after laparotomy, a left sided herniorrhaphy was planned with the horse in standing sedation using thoracoscopy. The 1st portal for the optical trocar (10mm) was located at the X intercostal space (ICS), pneumothorax was induced opening the trocar valve. Once identified the diaphragmatic defect 5 instrumental portals (1 of 10mm and 4 of 5mm) were located at the XIII and XIV ICS, 3cm dorsal and 3cm ventral to the 1st portal, and 2cm dorsal, 2cm ventral and 6cm ventral to the 1st portal respectively. A 10mm valveless trocar was used to insert the mesh. The non-absorbable three-dimensional polyester mesh provides long-term reinforcement of the soft tissue, while the opposite absorbable hydrophilic film minimizes tissue adhesion to the mesh in case of direct contact with the viscera. The polyester layer was laid on the diaphragm and fixed with coils (ProtacTM) and absorbable screws (SorbafixTM). After complete closure of the defect, the chest cavity was inspected and a negative thoracic pressure was reestablished.

Recovery was uneventful in the early days, but a septic pleural effusion developed that was treated with antimicrobials. After 30 days a second thoracoscopy was performed to evaluate the repaired hernia and to remove the sero-fibrinous material. The short term follow up showed a resolution of infection and no signs of recurrent colic.

DH is commonly congenital in foals whereas traumatic in adult horses1,5. Treatment includes conventional surgical intervention under general anesthesia in dorsal recumbency or thoracoscopically-guided surgery with rib resection in lateral recumbency2,4 or in standing thoracoscopy with suture material3. With an overall survival rate of 23% for all horses with DH and a success rate of 46%, the prognosis is generally guarded. Laparoscopic evaluation of DH, reduction of strangulated intestine, and surgical herniorrhaphy have been reported for small defects. The mesh adopted in this case was highly effective in the closure of the tear and it was easy to apply with the anchoring devices.

1)Collier DJ, Vet J 1999;31:358-359;
2)Malone et al, Vet Surg 2001;30:175-178;
3)Rocken et al, Vet Surg 2013;42;
4)Romero et al, Can Vet J 2010;51:1247-1250;
5)Santschi EM,Vet Surg 1997;26:242-245

MANAGING SECOND-INTENTION HORSE WOUNDS PRESENTING WITH EXUBERANT GRANULATION TISSUE USING A PLANT-DERIVED WOUND DRESSING: A RETROSPECTIVE NON-CONTROLLED STUDY.

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To evaluate the healing performances of traumatic horse wounds at the distal part of the limbs, presenting with Exuberant Granulation Tissue (EGT), using a plant-derived wound dressing (ONE©, Phytoceuticals, Zurich) in association, when feasible, with a permanent semi-occlusive bandaging.

A retrospective non-controlled study was conducted on 25 equine accidental wounds at the distal part of the limbs and treated with ONE© associated, when feasible, with permanent semi-occlusive bandaging, changed daily. Initial Wound Area (IWA - cm2) (calculated using a scaled digital photo or a wound contour traced on plastic film) and Time To Heal (TTH - days) were used for calculating the Epithelialisation Rate (ER=√IWA/TTH = cm/days - Stashak, 1991, Equine Wound Management, First Edition pp 1-18). The presence of the EGT was evaluated weekly using the Score System (EGT-SS ≤ 2 - Ducharme-Desjarlais et al. Am J Vet Res, 2005, 66, 1133-1139). Wound Appearance was recorded weekly as inflamed (≤3) or healthy (<3) on the basis of a scoring scale (WA score - Silveira et al. Am J Vet Res, 2010, 71, 229-234). Time of First Epithelium appearance (TFE - days) was evaluated weekly, Cosmetic Aspect of the final Scar (CAS score: 0= excellent; 1= good; 2= excessive scar - Ketzner et al. Austr Vet J, 2009, 87, 9, 368) was evaluated at the end of the healing process. Pain, complications, number of surgical EGT resections and ease of handling were recorded and evaluated.

The IWA mean size varied from 12,90 ± 4,51 cm2 (wounds <25 cm2) to 62,76 ± 26,55 cm2 (wounds >25 cm2). TTH showed a mean value of 79±54,32 days, ER was 0,0742±0,0342 cm/day and TFE was 18 days. Based on the EGT-SS, all of the 25 analyzed wounds presented EGT formation at the 15th day (EGT-SS ≤ 2) decreasing at <2 EGT-SS from the 30th day on. The WA score showed that at the 30th day all wounds reached a healthy wound state (<3) and no clinical signs of infection were observed during the whole remaining period, not even in those wounds in which bone was exposed (n=3). Bandaged Wounds (n=16) presented a better CAS score (88%=0, 12%=1, 0%=2) than Not Bandaged Wounds (n=9) (43%=0, 24%=1, 32%=2). No surgical resection was necessary, even if in the Not-Bandaged Wounds the wound surface slightly protruded the skin level. Horses became confident with medication without any sign of discomfort or pain over the whole time course.

Usually equine wounds presenting with EGT, have low healing performances and poor and disfiguring scar quality. The plant-derived wound dressing used in this study for treating the wounds shows the capacity to regulate the EGT formation, obtaining a high quality final scar, particularly when a permanent bandage is associated. It is simple to use and safe.

Stashak, 1991, Equine Wound Management, First Edition pp 1-18
Ducharme-Desjarlais et al. Am J Vet Res, 2005, 66, 1133-1139
Silveira et al. Am J Vet Res, 2010, 71, 229-234
Ketzner et al. Austr Vet J, 2009, 87, 9, 368

SECONDARY HYPERPARATHYROIDISM IN TWO PONIES.

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The purpose of this case series was to describe the historical, clinical, radiographic details, outcome and histhopathological
findings of 2 cases of NSH in pony.

CASE 1
A 3 years old, male pony was referred in 2003 for severe dyspnoea and deformity of the bones of the skull. At clinical examination, the pony had general fatigue, severe dyspnea and inspiratory breath sounds.
X-ray examination showed signs of severe thinning of the cranial bones with loss of definition of the plot medullary and cortical thinning cancellous bone. The results of parathyroid hormone by radio-immunological showed an increase in the values of PTH .Treatment has been provided using tiludronate (TILDREN 1mg/kg- ®-Ceva Vetem SpA, Milan). The patient's clinical condition showed significant improvements, the clinical symptoms (inability to maintain the station, lameness and respiratory sounds) had resolved. The pony was dismissed.

CASE 2
In October of 2012 was referred a 5 years old Shetland male pony, and the owners permanent recumbency since 3 days.General clinical examination showed persistent recumbency , slight enlargement of facial bones and pain at flexion of hindlimbs. Based on the clinical symptoms, X-rays and laboratory tests, the diagnosis was of NSH related to fibrous osteodystrophy.
The clinical condition of the horse showed no improvement and the subject was not able to take quadrupedal station. The owner disagrees with Tiludronate therapy and pony was euthanized, and some bone samples were harvested in order to deepen the diagnostic picture.

Microradioghaphs of frontal serial sections show modifications of bone tissue arrangement and mineralization.. The trabecular network appears rarefied owing to a thinning of the trabeculae or their total erosion. Both spongy and compact bone frequently exhibit large osteocytic lacunae. On the other hand new bone, less mineralized, is exthensively present. The deposition fronts show a wide osteoid with voluminous osteoblast, but sometimes exhibit numerous preosteoblasts with a fibroblasts morphology. In many area, fibrous connective tissue covers the trabecular surfaces and replaces part of the marrow reticular stroma.

The appearance of micro X-ray, never described in the horse, and the histological evidence and confirm the diagnosis of fibrous osteodystrophy.
Bisphosphonate therapy is a therapeutic option, and it is valid for the possible remission of clinical symptoms but not certain deformities of the disease. The association of such therapy with a balanced diet can be part of the treatment protocol in the course of NSH of dietary origin.

David et all., the bisposphonate Tiludronate is a potent inhibitor of the osteoclast vacuola hatpase, Journal of Bone and Mineral Research, 11, 1498-1507, 1996

Estepa, J. C., Aguilera-Tejero, E., Mayer-Valor, R., Almeden, Y., Felsenfeld, A.J. & Rodriguez, M. Measured of parathyroid hormone in horses. Equine Veterinary Journal 30, 476-481, 1998

RECURRENT BLADDER UROLITHIASIS IN THE HORSE: A CASE REPORT.

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Aim of this study is to report a case of recurrent bladder urolithiasis in the horse, stressing the importance of early diagnosis by flexible videoendoscopy.

Italian saddle horse, 17 year old, neutered, chestnut coat, admitted to the Military veterinary hospital in February 2013 because of hematuria/stranguria and suspected recurrent urolithiasis (in 2008 the same patient underwent the surgical removal of a bladder urolith with spiculated surface, elliptical shape and about 7 cm of diameter). Clinical exam, focused on transrectal palpation, demonstrated a solid and mobile mass in the bladder. Transrectal ultrasound exam (Esaote "Mylab one ™" , 5-7.5 MHz linear probe) and cystoscopy (STORZ PV-SG22-140 flexible videoscope) confirmed the presence of a bladder urolith with elliptical shape, spiculated surface, dimensions of 6,5x4x3,5 cm. and partially adherent to the mucosa. The horse underwent surgery to remove the urolith by laparocystotomy technique through the right parapreputial access.

75% of urolithiasis cases in horses are referred to males. The reason behind this incidence is that a mare has a shorter and distensible urethra that permits voiding of small calculi. The 60% of uroliths are most common in the urinary bladder. For uroliths to develop, two steps are necessary: nucleation and crystal growth. Horses develop two forms of uroliths, both composed by CaCO3. Most of these are spiculated and can be easily fragmented (type I urolith), as in the case described. Rarely, uroliths are gray-white, smooth and more resistant to fragmentation (type II urolith). In the case of urolithiasis, the treatment of choice includes surgical removal through laparocystotomy and laser lithotripsy: the choice between different methods is based on size, nature, friability of the calculus and sex of the horse. An incomplete removal of a type I urolith may cause relapses as the fragment that remains in the bladder may constitute itself a new nucleus of formation. The control of inflammation of the urinary tract and the administration of acidifying substances in the postoperative period are to aid in the prevention of relapses. In order to exercise a proper preventive action, an adequate diet is important as well to reduce the excretion of calcium and stimulate diuresis (Reed SM et al., 2010).

Modern flexible videoscope (about 9 mm diameter /140 cm length) confirms to be a useful means for the diagnosis of urolithiasis in the horse, and for the endoscopic removal of small uroliths. Furthermore this method, low risk if carried out by an experienced operator, could be used for the prevention on those subjects whose clinical history predispose to the disease.

Reed SM, Bayly WM, Sellon DC, editors: Equine Internal Medicine, ed 3, St Louis, 2010, WB Saunders.

IN VITRO IMMUNOMODULATORY ACTIVITY OF CONDITIONED MEDIUM OBTAINED FROM AMNION-DERIVED HORSE PROGENITOR CELLS AND ITS FIRST CLINICAL APPLICATION IN HORSE TENDON INJURIES

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We recently demonstrated that heterologous transplantation of horse amniotic membrane-derived mesenchymal cells (AMCs) is useful for cell therapy applications in tendon diseases (Lange-Consiglio et al. 2012,2013). Whether MSCs differentiate into tenocytes, supply immunomodulatory and trophic factors or if a combination of the two mechanisms occurs, is still debated. To test this hypothesis, we examined the immunomodulatory characteristics of AMCs and of their conditioned medium (AMCs-CM) in vitro, and studied the therapeutic effect of AMCs-CM in horse tendon injuries in vivo.

To produce AMCs-CM, AMCs at passage 3 were cultured for 5 days. Supernatants were lyophilized and stored at 4°C until use. Control (non-CM) was generated in the same way but without cells culturing. Lymphocyte proliferation was induced by stimulating peripheral blood mononuclear cells (PBMC) by phytohemagglutinin at the concentration of 2 µg/ml. To evaluate the effect of AMCs-CM, 50 or 100 µl/well of this supernatant or no-CM were added to activated PBMC. Effects of AMCs were studied either by cell-cell contact or by transwell system maintaining constant the number of PBMC (2*105) and decreasing the number of AMCs, to obtain ratios of PBMC:AMCs of 1:1, 1:0.5, 1:0.25, 1:0.125.
Sterile CM was intralesionally injected under ultrasonographic guidance in spontaneously damaged tendons of 13 private sport horses. Patients were clinically and ultrasonographically monitored monthly. Success criteria was return to former athletic function and absence of relapses.

Results demonstrated that AMCs are capable of inhibiting PBMC proliferation in a dose-dependent manner, either in cell-cell contact or in transwell system reaching a 90% ($P \leq 0.05$) decrease of PBMC proliferation at a ratio of 1:1. The same effect was also observed for the AMCs-CM but not for the control media. In vivo, intralesional procedures were well tolerated and a marked reduction in swelling and tendon cross sectional area were noticed. A treatment related neovascularization was Power Doppler imaged, in the affected area, in the early healing phase but not at later recovery stage. An obvious improvement in lesional ecogenicity and architecture was clearly noticeable just after 30 days. Two years after CM injection 84% of horses showed no relapses.

Our findings suggest that soluble factors are implicated in inhibiting PBMC proliferation and in tendon regenerative process that may initiate an anti-inflammatory and angiogenic response leading to tendon regenerative process. CM should be considered a safe, novel biologic cell-free therapeutic agent in regenerative medicine.

1. Lange Consiglio et al. Characterization and potential applications of progenitor-like cells isolated from horse amniotic membrane. J Tis Eng Reg Med 2012; 6: 622. 2. Lange Consiglio et al. Investigating the efficacy of amnion-derived compared with bone marrow-derived mesenchymal stromal cells in equine tendon and ligament injuries. Cytotherapy epub 2013.

VALIDATION OF TISSUE MICROARRAY FOR CANINE AND FELINE MAMMARY TUMORS MOLECULAR PROFILING

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Tissue Microarray (TMA) is a high-throughput method, adopted for simultaneous molecular profiling of tissue samples from large patient cohorts. The aim of this study is to validate the TMA method for molecular classification of canine (CMT) and feline (FMT) mammary tumors.

Ten cases of CMT and FMT and 2 cases of canine hemangiosarcoma were collected. A sector map, which specifies a location within the tissue array for each core sample, has been designed. The process is based on Kononen's method of extracting a cylindrical core of paraffin-embedded donor tissue and inserting it into a recipient paraffin block. In Tissue Array Blocks of CMT and FMT cores, 1 core of canine hemangiosarcoma was used as landmark. Cores, 4 mm in diameter, were extracted from an empty pure paraffin block, indicated as Recipient Block (RB). The tumor area was selected in the glass slide and then compared with the corresponding paraffin embedded tissue block, Donor Block (DB). The tumor core was extracted in order to be re-embedded in the empty cylinders previously created in the RB. After array construction, a section was cut for Hematoxylin-eosin staining to perform a quality control. The qualitative (representative tumor tissue) and quantitative (number of tumor cells) content of each core was assessed. Seven consecutive sections from each Tissue Array Block were subjected to immunohistochemistry (IHC) using antibodies anti-ER, -PR, -c-erbB-2, -CK5/6, -CK14, -CK19 and -p63. The same panel of antibodies was applied to the full sections (FSs) of all cases to compare the immunohistochemical results between FSs and TMAs.

Comparison between FSs and TMAs score revealed different results depending on the antibodies. ER, PR, CK19 and p63 showed a total concordance between FSs and TMAs. Positivity of c-erbB2 (score +2, +3) were concordant in 8 out of 10 cases between FSs versus TMAs. CK5/6 stained positive in 7 of 10 FSs and in 5 of 10 TMAs cores, with loss of expression in 2 cases. All FSs were positive for CK14, with loss of expression in 2 of 10 cores of TMAs.

TMA platform preserves the molecular profile of CMT and FMT markers, representing a useful tool for rapid and cost-effective analysis for the first phenotypic screening, provided by the use of ER, PR and c-erbB2. Basal cytokeratin, used for triple negative identification, shows a multifocal “niche” expression pattern, for which the IHC of the full section is recommended.

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HISTOMORPHOMETRIC PARAMETERS AND FRACTAL COMPLEXITY OF THE EQUINE PLACENTA FROM HEALTHY AND SICK FOALS

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The aim of this study is the investigation of histomorphometric parameters and fractal complexity of the equine placenta from healthy and sick foals via digital image analysis (DIA).

Fourteen placentas out of 40 mares were selected and divided into 2 groups: 7 mares with normal pregnancy, eutocic delivery and healthy foal (group H); 7 mares with normal or high-risk pregnancy, eutocic delivery and sick foal (group S). Samples for histopathology were obtained after gross evaluation from three placenta segments: body (B), gravid horn (GH) and non-gravid horn (NGH). DIA was performed with ImageJ software. Histomorphometric measured features were: number of villi, total area of villi, percentage of filled area, mean area of villi, mean perimeter of villi, circularity, solidity and fractal dimension (Fd).

Mean Apgar score was 9 in group H and 8 in group S. Four out of 7 sick foals were affected by Perinatal Asphyxia Syndrome, 2 by prematurity/dismaturity; 1 by both. The following statistical differences were found with U-Mann-Whitney test between the 2 groups: B - number of villi, circularity, and solidity ($p<0.05$); GH – total area of villi, percentage of filled area ($p<0.05$), and circularity ($p<0.01$); NGH – number of villi, Fd ($p<0.01$), total area of villi, and percentage of filled area ($p<0.05$). In samples obtained from B and GH, values were higher in healthy than in sick foals, whereas in NGH values were higher in sick than in healthy foals. By Spearman's test Apgar score was associated with: GH - mean area of villi (R -0.638, P 0.014), mean perimeter of villi (R -0.608, P 0.021); B - circularity (R 0.583, P 0.029), solidity (R 0.669, P 0.009), Fd (R -0.550, P 0.042); NGH – Fd (R -0.538, P 0.047); mean value of Fd in the 3 segments (B+GH+NGH) (R -0.564, P 0.036).

Results of this study point out that in sick foals chorionic villi tend to have a higher branching and complexity than in healthy ones, particularly in non-gravid horn. This result could suggest an attempt to increase the exchanging area between fetal and maternal compartments. It is further supported by the correlations between Apgar score and some histomormophetric parameters and Fd, since a low Apgar score indicates neonatal hypoxia.

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SEMEN COLLECTION AND EVALUATION IN COLUMBA LIVIA

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Despite CASA system has been successfully used in human and veterinary medicine, avian semen has been evaluated much less often with this method (1). The objective of this study was to assess the effect of dilution on pigeon semen motility parameters, to evaluate spermatozoa survival at 37.5C° and to evaluate the effect of chilling at 4°C.

Semen was collected from three adult males by sacral and addominal-cloacal massage (3). Each ejaculate, after volume calculation by a calibrated pipette, was immediately 1:5 diluted in M199 no-Hepes (Sigma-Aldrich). After a second dilution (1:20) at 40°C, 10 µl of the final sample were transferred into a Makler chamber and concentration and motility parameters were measured using CASA (CEROS, Hamilton Thorne Research Inc., Version 14). Semen was further diluted in M199 at 1:50 and 1:100 and analyzed. 1:100 diluted samples were incubated at 4 and 37.5°C and motility was recorded hourly for 8 hours. The effect of dilution was analysed with factorial one way ANOVA (Bonferroni post-hoc test); the effect of incubation at different temperatures (4° and 37°) was analyzed using factorial one way Anova for Repeated Measure (SPSS® ver.19).

30 ejaculates were collected; semen volume ranged from 5 to 20 µl. Mean spermatozoa concentration was 4.14±0.23 x 109ml-1, mean motile spermatozoa were 2.40±0.22 x 109ml-1 and 57.48±18.6 x 106ml-1/ejaculate. Dilution significantly affected some motility parameters, VAP, VSL, STR, LIN (Tab. 1), which were higher at increasing dilution; D20 resulted worse than D:50 and D:100, while no difference was found between D:50 and D:100. Also total motility was numerically higher at higher dilutions ($p=0.08$) while no effect was seen on progressive motility ($p=0.38$). When semen was incubated at different temperatures, a time effect was observed both in total and progressive motility ($p<0.01$; $p=0.001$), while temperature did not affect either motility parameter ($p= 0.51$; $p= 0.63$) (Tab. 2). At time 0, freshly diluted pigeon spermatozoa were extremely active, progressively motile, with linear trajectories. By 8 h of incubation at 37.5°C, motility dropped at about 2% (Tab. 3).

This study was carried out in March. About 90% of collection attempts yielded an ejaculate and mean spermatozoa concentration was similar to literature data (4, 1). The extender M199 no-Hepes gave promising results and semen quality was higher than what found in previous works (1), in which different extenders were used. Dilution significantly affected semen quality, with higher dilutions giving better results. The CASA system resulted useful to analyze pigeon spermatozoa. Further studies are necessary to better understand pigeon semen characteristics (pH, osmolarity) and to define the minimum fertilizing dose.

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Dilution	% Mot.	% Prog.	VAP	VSL	STR	LIN
D20	22.9 ± 17.8	11.4± 7.8	80.8 ± 14.0	68.8 ± 16.7	80.6 ± 10.2	55.1 ± 13.7
D50	38.4 ± 20.2	25.8 ± 16.3	109.7 ± 31.0	101.1± 30.8	87.8 ± 6.0	68.4 ± 10.6
D100	23.61±17.7	17.3 ± 15.0	100.2 ± 25.5	94.0 ± 25.8	89.4 ± 4.9	69.2 ± 8.8
p-value	0.08	0.38	0.05	0.03	0.02	0.013

Table 1: Effect of semen dilution (1:20, 1:50, 1:100) on motility parameters of Blue rock pigeon spermatozoa (means ± SD)

	% Mot.	(T0)	(T1)	(T2)	(T3)	(T4)	(T5)	(T6)	(T7)	(T8)
4 °C	D	31.1±12.3	21.5±10.1	11.4±6.9	8.2±4.8	5.5±2.8	5.6±5.4	3.1±2.6	3.1±3.8	2.5±3.9
37.5°C	D	68.5±12.4	7.2±3.3	6.4±5.2	15.0±12.9	8.9±8.2	6.7±5.9	4.2±7.0	3.8±6.6	2.2±6.3
	% Prog.									
4 °C	D	40.4±32.6	14.0±7.7	5.8±5.2	4.2±3.7	2.5±1.8	3.1±3.8	1.5±1.3	1.7±2.1	1.2±2.0
37.5°C	D	24.2±10.0	2.8±1.6	2.6±1.8	9.6±9.4	4.1±4.6	2.1±2.1	2.0±3.6	1.8±3.0	0.8±2.1

Table 2: Effect of incubation at different temperatures (4°C and 37.5°C) on Blue rock pigeon spermatozoa motility parameters over 8 hours

	VAP	VCL	VSL	STR	LIN	ALH	BCF
T0	102.9±27.0	131.8±22.4	95.9±27.6	89.0±4.2	68.1±8.9	4.6±0.4	32.1±4.2
T1	65.0±15.4	99.6±15.7	56.0±14.9	78.9±13.9	53.7±6.6	4.9±0.5	28.8±3.2
T2	57.0±15.4	89.5±15.9	47.4±16.0	79.8±6.3	51.7±7.6	4.6±0.7	27.4±3.8
T3	67.0±17.9	100.3±17.4	57.4±18.6	81.8±6.4	55.3±10.1	5.1±1.1	27.9±4.4
T4	56.5±10.6	91.3±12.2	46.1±11.7	77.8±8.2	49.7±7.3	4.9±0.8	29.0±3.6
T5	48.1±27.6	76.8±40.8	39.4±24.3	63.4±32.2	41.0±22.1	3.4±2.3	21.4±11.4
T6	37.6±31.0	61.7±47.8	30.8±25.7	48.8±39.8	32.1±26.6	2.9±2.4	17.1±14.2
T7	28.8±32.3	51.2±33.9	24.9±28.3	39.1±42.1	26.1±28.3	2.4±2.6	13.6±14.8
T8	17.0±28.3	26.5±43.6	14.3±24.4	22.8±37.2	15.0±24.6	1.4±2.4	7.3±12.2

Table 3: Time-dependent motility parameters of Blue rock pigeon spermatozoa incubated in M199 at 37.5°C (means ± SD) (total and progressive motility are reported in Table 2)

PLASMA T3 AND T4 CONCENTRATIONS IN NEWBORN MARTINA FRANCA DONKEY FOALS

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In the complex neonatal adaptation to the extra-uterine life, thyroid hormones play an important role in the process of growth, and in energy, nutritional and mineral metabolism, as well as in thermogenesis. Thyroid hormones profiles, widely studied in human newborns, have been also studied in horse foals (1). The aim of the present study was to evaluate the T3 and T4 plasma concentrations in healthy newborn Martina Franca donkey foals, from birth to 14 days of age.

The study was conducted on 6 Martina Franca donkey foals, born by 4-19 years old, 2-8 parity jennies. Foals were evaluated for gender, birthweigth and viability, assessed by Apgar score measured within 5 minutes after birth and by the intervals between birth and stand up (TSU) and to first suck (TFS). Blood samples were collected by jugular vein at 10 min after birth, at 12 hours and at 3, 7, 10 and 14 days of age and obtained plasma stored at -20° C until T3 and T4 analysis by RIA (1). One-way ANOVA was used to assess possible changes in both hormones among the sampling times.

The 6 foals, 3 females and 3 males, were born at term after 369 ± 3.11 days of gestation, and foalings were spontaneous and eutocic. The foals were healthy, mature, with normal birthweigth (30-35 kg), and viable (Apgar >8; TSU 63.8± 9.73 minutes, TFS 105 ± 16.9 minutes). Jennies gestation length as well as the donkey foals characteristics were within the normal ranges for the donkey (2). Mean (±SD) plasma T3 and T4 concentrations in the 6 foals, from birth to 14 days of age are reported in table 1.

In donkey foals the T3 and T4 profiles showed a decreasing trend from birth to the 14 days of age, as previously reported for the horse foal (1). Taking into account the high inter-individual variability, T3 and T4 plasma levels decreased significantly from 3 days of age to 10 and 14 days. The study evidenced that also in donkeys, T3 and T4 are involved in the process of neonatal adaptation and that their profiles are similar to what reported for the horse foal.

1) Panzani et al, 2012 Theriogenology, 77, 1167-1177; 2) Panzani et al, 2012, Reprod Dom Anim, 47, 82-86.

Table 1 – Plasma T3 and T4 concentrations (mean ± SD) concentrations in the 6 Martina Franca donkey foals, from birth to 14 days of age.

Age	T3 (nmol/l)	T4 (nmol/l)
	Mean ± SD	Mean ± SD
10 min	13.1±1.35^{ab}	541.41±155.18^{a}
12 hours	19.71±6.04^{a}	450.24±152.94^{a}
3 days	20.05±6.79^{a}	316.74±144.9^{b}
7 days	9.42±4.8^{c}	121.52±99.30^{c}
10 days	6.28±2.38^{c}	70.83±42.72^{c}
14 days	4.71±3.25^{c}	57.22±40.53^{c}

Different superscript within column refers significant differences (P<0.05)

THE ECONOMIC IMPACT OF THE REMOTE CALVING MONITORING IN THE DAIRY INDUSTRY

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In the dairy industry, calf delivery and newborn calf management are often undervalued as areas of concern, due to the costs of calving monitoring. The postpartum period is a crucial moment for the future reproductive performance of dairy cows. The profitability of dairy farms depends greatly on the reproductive efficiency of dairy cows [1].
Several protocols have been proposed to determine the exact moment of the calving, such as ultrasound monitoring, hormonal and electrolytic levels analysis; unfortunately these protocols allow the start of calving to be predicted within a window of several hours, requiring frequent monitoring of cows [2]. The remote calving monitoring approach suggested in this study gave an accurate determination of the beginning of the second stage of labor.
The aims of the study were to: a) appraise economic value of the calving monitoring through the use of the remote alarm system, b) determine if the cost of post-partum reproductive pathologies and stillbirths could be reduced through prompt emergency obstetric procedures, thus leading to an improvement of herd fertility in a dairy farm in Central Italy.

The incomes were represented by the milk produced within 60 days in milk, the calf value and, in case of culling, the value of the carcass. Expenses were produced by post-partum diseases, artificial inseminations, other treatments, milk discarded value, transport and slaughter, carcass disposal and the value of dead heifer or multiparous.

Heifers assisted during the calving (n = 60) have shown an average income of € 796.31 vs. the profit of € 815.84 from the unassisted heifers (n = 269). The net losses from these groups were € 48.46 vs. € 192.99, for a net gain of € 747.85 per monitored heifer vs. € 622.85 per unmonitored primipara. The difference was more evident among multiparous cows. Profits amounted to € 916.54 from assisted pluripara (n = 60) vs. € 792.57 from unmonitored cows (n = 172). The losses produced reached € 113.87 in the assisted group vs. € 249.43 in the unassisted one, for a net gain of € 802.67 per cow in the monitored group vs. € 543.14 per unmonitored cow.

In conclusion, calving monitoring strategy appears to improve the productive and reproductive efficiency of the dairy herd and is a relevant economic factor for the dairy industry, allowing a reduction in the incidence of post-partum pathologies and stillbirths, and a decrease in costs for treatments and amortizations.

[1] Meadows C et al J. Dairy Sci. 2005;88:1244 –1254
[2] Paolucci M et al Vet Res Commun 2010; 34 Suppl.1:S37-40

USE OF A TISSUE SEALING DEVICE FOR GONADECTOMY IN CATS

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In recent years, advances in technology, together with the development of new surgical techniques, particularly those of laparoscopic surgery, have led companies to develop more sophisticated systems for tissue dissection and coagulation (1). Among these, the system ENSEAL®, which combines the effect of compression and temperature, associating also the possibility of dissecting the tissue after vessels coagulation. The specific technology allows a control of temperature at the tissue level which remains constant at around 100° C, while the peculiar type of electrodes also limit the spread of energy outside the jaws, limiting to few millimeters the area were tissue temperature increase. In addition, the position of the blade allows to maximize the pressure on the tissue at the time of cutting. Aim of the study was to test the properties of this device for routinary gonadectomy in the cats.

The instrument has been used for the gonadectomy of 40 cats (20 males and 20 females), at the Veterinary Hospital of the University of Padua and in a private clinic. Anesthesia was induced with i.m. injection of medetomidine 0.02 mg/kg, ketamine 10 mg/kg and methadone 0.2 mg/kg. In females oxygen was provided by tracheal intubation, while in males the oxygen was administered by mask. In both sexes, when the traction on the gonad gave an increase in heart rate it was provided a “splash” with 0.2 ml of 2% lidocaine on the spermatic cord or on the ovarian pedicle. The instrument was applied on the spermatic cord in the male and on the ovarian pedicle and at the utero-ovarian junction in the female. After about 10 seconds the instrument signaled that the coagulation took place; after an inspection of the site of coagulation the instrument was applied again and at the next signal that coagulation occurred the dissection was carried out.

In none of the cases bleeding occurred after resection; during the application of the instrument there were no changes in the heart rate (range 90-120 bpm) and the respiratory rate (range 5-8 RR). Histological examination of the stump of a spermatic cord testifies for a coagulative necrosis. Post surgery, animals treated more solito showed no significant alterations.

In conclusion, the use of this instrument showed advantages, the procedure, fast and easy, permit to avoid the use of suture material inside the abdomen and does not show negative effects for the patient.
Of course, aspects relating to the cost of the device are to be considered because, despite having adopted a system of effective re-sterilization, a significant investment is required.

1) A Comparison of Laparoscopic Bipolar Vessel Sealing Devices in the Hemostasis of Small-, Medium-, and Large-Sized Arteries. A.M. Carbonell, C.S. Joels, K.W. Kercher, B.D. Matthews, R.F. Sing, B.T. Heniford. Journal of Laparoendoscopic & Advanced Surgical Techniques. December 2003, 13(6): 377-380

IGF-I AND NEFA CONCENTRATIONS IN FETAL FLUIDS OF TERM PREGNANCY DOGS

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IGF-I and NEFA are listed among the factors playing a role in fetal growth and development in mammals. In humans IGF-I and NEFA amniotic concentrations differ between normally developed fetuses and those affected by intrauterine growth retardation (IUGR) [1,2], but they were not investigated in dogs. The present study was aimed to evaluate IGF-I and NEFA levels in fetal fluids of healthy bitch at term pregnancy and to assess possible differences according to body size.

The study enrolled 25 bitches, belonging to several breeds, submitted to elective cesarean section at term. The amniotic and allantoic fluids were collected aseptically from each puppy and stored, after centrifugation, at -20°C until analysis by RIA for IGF-I [3] and by enzymatic-colorimetric methods for NEFA [4]. At birth, the newborn puppies were evaluated for viability by Apgar score [5], maturity, sex, absence of gross malformations, and weight. IGF-I and NEFA concentrations in both fluids were evaluated by one way ANOVA followed by Fisher's LSD test.

Only viable, mature, healthy, and with normal birth weight puppies were enrolled. On the basis of bodyweight, the 25 bitches were divided into 3 groups: small, medium, and large size. Mean (±SD) amniotic and allantoic IGF-I and NEFA levels, measured on a total of 73 amniotic and 76 allantoic samples belonging to the 25 litters, are reported in Table 1.

In dogs at term, IGF-I concentrations are significantly higher in amnion collected from puppies belonging to large breeds compared to small and medium, suggesting that IGF-I could be an indicator of growth potential in dogs as previously suggested [6]. In both fluids NEFA levels are significantly higher in small breeds than medium and large, as reported in case of IUGR in humans [2] .

1. Delmis J et al. J Perinat Med 1992; 20:47-56. 2. Urban J et al. J Perinat Med 1986; 14(4):259-62. 3. Renaville R et al. J Reprod Fertil 1993; 99:443-9. 4. Accorsi PA et al. Reprod Dom Anim 2005; 40:217-223. 5. Veronesi MC et al. Theriogenology 2009; 72: 401-07. 6. White ME et al. Am J Vet Res 1999; 60:1088-91.

Table 1- Amniotic and allantoic IGF-I and NEFA levels (mean±SD) in the 25 litters, divided in small, medium, and large size.

	IGF-I (ng/ml)		NEFA (ng/ml)	
Bitches (25)	Amniotic fluid (73)	Allantoic fluid (76)	Amniotic fluid (73)	Allantoic fluid (76)
Small: ≤10 kg (16)	32 ± 13.21^{a} (38)	23 ± 15.01 (41)	44 ± 34.80^{a} (38)	38 ± 27.04^{a} (41)
Medium: 11-25 kg (4)	29.8 ± 13.19^{a} (18)	26.5 ± 18.54 (16)	22.7 ± 10.99^{b} (18)	36 ±18^{b} (16)
Large: 26-40 kg (5)	43.6 ± 11.46^{b} (17)	19.6 ± 9.49 (19)	14.8 ± 10.74^{b} (17)	11.9 ± 11.22^{b} (19)

^{a,b}Different superscript within column refers significant differences ($p<0.05$)

EVALUATION OF MEAN ECHOGENICITY OF TENDONS AND LIGAMENTS OF METACARPAL REGION IN FOALS: PRELIMINARY REPORT

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To evaluate Mean Echogenicity (ME) of deep and superficial digital flexor tendons (DDFT and SDFT), suspensory ligament (SL) and inferior check ligament (ICL) in 3 to 10 days-old foals and to determine the effect of sex and laterality (left and right limb) on ME.

Right and left metacarpal regions (palmar surface) of 12 ortopedically sound foals of different races and 3-10 days of age were examined. The study was performed on standing foals with a portable real-time ultrasound scanner (Esaote My Lab One) with a 10 MHz linear probe. Skin and hair were washed with warm water; ethyl-alcohol was applied. Four areas of study (1A, 1B, 2A, 2B) were identified, according to the Rantanen classification (1), adapted to the foal reducing each zone width to 2 cm. Transverse scans of DDFT, SDFT, SL and ICL were obtained. Ultrasound images were digitized and ME was calculated using a dedicated software (ImageJ; Image Processing,USA) with a scale of 256 grey levels. A blind evaluation with two expert investigators was performed. Intra- and inter-observer reproducibility was estimated by Bland-Altman and Spearman methods. ME differences between examined structures were analyzed using an ANOVA test ($p \leq 0.05$). The influence of gender and laterality on echogenicity was also analyzed by using the Student's t-test ($p \leq 0.05$).

All 12 foals (6 males and 6 females) met the inclusion criteria. None foal was sedated during examination. Reproducibility and agreement tests between operators gave a result of 0.8 (very good). In relation to ME, the ICL and DDFT were the most echogenic structures, whilst the SDFT was significantly less echogenic than all other structures ($p < 0.05$). There was no significant difference in ME between males and females and between right and left forelimb.

The importance of ultrasonographic measurement of ME in adult dog and horse has been well established (2,3,4), but to the authors' knowledge there are currently no publication containing information regarding quantitative ultrasonographic tendon measurements in neonatal foals. The study has emphasized a scale of echogenicity of the teno-desmic structures comparable to that reported in adult horses (1,3,4), as well as a lack of influence of sex and laterality on ME in the first 10 days of age(4). Cellularity and fiber undulation observed in SDFT of the very young foals could be the reason of lesser echogenicity of this tendon(5). A limit of this study was the restricted number of foals, which would be extended to create a more reliable database in order to discriminate between normal and pathological conditions.

1)Sande RD et al.In: Equine Diagnostic Ultrasonography. Williams and Wilkins ed 1998, 103-123 – 2)Spinella G et al, Vet Comp Orthop Traumatol 2013, in press – 3)Van Schie HT et al, Am J Vet Res 2000, 61: 210-219 – 4) Agut A et al, Vet J 2009, 180: 377-383 – 5)Crevier-Denoix N et al, Am J Vet Res 1998, 59: 969-977.

PLASMA T3 AND T4 CONCENTRATIONS IN NEWBORN CALVES: INFLUENCE OF TYPE OF DELIVERY AND BREED

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In the neonatal adaptation to the extra-uterine life, thyroid hormones play an important role in the process of growth, energy metabolism and thermogenesis. Thyroid hormones profiles were described in newborn calves from different breeds (1), while the influence of gender, birth weight and type of delivery is still unclear. The aim of the study was to compare T3 and T4 plasma concentrations in Holstein-Friesian newborn calves born by vaginal delivery and Belgian Blue born by caesarean section.

The study was conducted on 12 Holstein-Friesian calves born by vaginal delivery (VD) and on 12 Belgian Blue calves born by caesarean section (CS). Calves gender, body weight and viability were recorded immediately after birth. Blood samples were collected from jugular vein at 10 and 20 minutes (m), at 6 and 24 hours (h) and at 7 and 14 days (d) of age and plasma stored for T3 and T4 analysis by RIA. The ANCOVA for repeated measures test was used to assess the effect of gender and birth weight on T3 and T4 plasma levels profiles within each group. One-way ANOVA was used to evaluate possible differences in T3 and T4 levels between the two groups in each sampling time ($p<0.05$).

All newborn calves were mature and viable. Mean body weight was 34±4.2 Kg for VD (5 females and 7 males) and 53±7.7 Kg for CS group, respectively (5 females and 7 males). Means ± SD of T3 and T4 plasma concentrations in the two groups of calves are reported in table 1.

In both groups the T3 and T4 profiles showed an increasing trend from birth to 6 hours of age, followed by a decrease to 14 days of age, as previously reported (1). According to statistic results, T3 and T4 plasma levels were not affected by gender and by birth weight. Statistics evidenced higher T3 and T4 concentrations in the CS calves early after birth, possibly due to the faster process of birth that may result in an immature T4 deiodination system in Blue Belgian calves. Higher plasma levels of both hormones were also detected in the CS calves at 7 and 14 days of age; differences in nutrition or in body weight gain could be responsible for these differences. Unfortunately the association between different type of delivery and different breed within each group does not allow to clarify the real effect of these variables on T3 and T4 plasma levels in newborn calves.

1) Davicco et al, 1982 Reprod Nutr Dev 22, 355-362

Table 1. Means±SD of T3 and T4 plasma concentrations in newborn calves from VD and CS.

		Sampling times					
		10 min	20 min	6 h	24 h	7 d	14 d
T3	VD	1.17±0.43^{a*}	3.52±1.19^{a}	7.16±2.93^{b*}	8.43±1.68^{b}	1.24±0.52^{ac*}	1.27±0.82^{ac*}
	CS	1.38±0.63^{a*}	3.57±1.63^{a}	10.47±3.01^{b*}	6.83±1.19^{b}	2.63±0.92^{ac*}	2.40±0.84^{ac*}
T4	VD	17.34±11.23^{a*}	150.14±65.99^{a}	205.77±54.88^{b}	196±46.25^{b}	31.86±13.2^{ac*}	39.84±17.27^{ac*}
	CS	35.3±15.7^{a*}	165.93±42.41^{a}	208.1±28.33^{b}	168.21±40.1^{b}	60.89±22.11^{ac*}	66.22±16.51^{ac*}

Different superscript within rows refers to significant differences within the group ($p<0.05$); * within column refers to significant differences between groups in each sampling time ($p<0.05$)

EVALUATION OF NEONATAL VITALITY IN BOVINE

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To determine the usefulness of clinical and hematic parameters in the evaluation of newborn calf vitality, in order to identify subjects requiring timely care.

21 Friesian calves have been enrolled in the study, in accordance with D.L. 116/92 and after approval by Ethics Committee of Bologna University and Italian Ministry of Health. Immediately after delivery (T0), calves were subjected to clinical examination (Body Temperature, BT; Body Weight, BW; Time to Suckling Reflex, SR; Time to Sternal Position, SP; Time to Standing, TS) and APGAR score's evaluation (range 0-2 for each parameter and 0-10 for the total score; 1) (Table 1). Clinical examination was repeated 24 hours after parturition (T24). Blood glucose concentration (BG; mg/dL) was evaluated at T0 and T24. Blood lactate concentration (BL; mmol/L) was evaluated at T0, T24 and T48. Serum total protein (TP) was evaluated at T24 to verify passive transfer of immunity. The trend of BL and BG was analyzed by one-way ANOVA. The correlation between considered parameters was performed using Spearman test. Results were considered statistically significant for $p<0.05$.

Twenty calves were healthy and in good condition at birth. Results are reported in Table 1 and 2. Only one male calf was sick immediately after birth and, 20 hours later, was subjected to euthanasia; therefore, it was not included in statistical analysis. Positive correlations, resulted from the study of considered parameters, are reported in Table 3.

Also in bovine, as previously reported in other species, lactate decreases significantly 24 hours after birth, when the adaptation to extra-uterine life should be completed.
If these preliminary results are confirmed by further studies, on sick and healthy calves, the evaluation of blood lactate and APGAR score may be an useful aid for early detection of calves requiring timely care at birth.

1 Vaala WE; House JK, Madigan JE. Initial management and physical examination of the neonate. In: Smith PB editor. Large Animal Internal Medicine, St Louis Mosby, 2002; pp 277-293.

	Mucous membranes	Pulse	Nose/ear stimulation	Muscular Tone	Respiration	Total Score
Mean value	1.8±0.8	1.9±0.6	1.9±0.4	2.0±0.0	2.0±0.2	9.4±0.8

Table 1. APGAR score mean value registered 5-10 minutes after birth.

Parameter	T0	T24	T48
SR (min)	5±2.8	/	/
SP (min)	3.5±1.7	/	/
TS (min)	32.8 ± 20.3	/	/
BT (°C)	38.8 ± 0.4[a]	38.4 ± 0.3[a]	/
BL (mmol/L)	5.5 ±3.2[b]	3.5 ±1.7[c]	2.9 ±1.0[c]
BG (mg/dL)	72.3 ±32.2[d]	125 ±26.4[e]	/
TP (g/dL)	/	5.2±0.7	/

Table 2. Clinical and biochemical mean values recorded at T0, T24 and T48 (b vs c, d vs e: $p<0.05$).

Parameter	n. of samples	R	p
TP-APGAR	20	0.504	0.002
TP-SR	20	0.658	0.001
SR-BG T0	20	-0.451	0.05
BG T24-BW	20	0.445	0.05
BW-APGAR	20	0.508	0.02

Table 3. Statistical significant correlations between considered parameters.

IN VIVO PERFORMANCE OF BUFFALO YOUNG BULLS FED FABA BEAN AS PROTEIN SOURCE IN DIET

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To reduce feeding costs and favour the domestic production of GMO-free plant proteins several farmers attempt protein sources alternative to soybean (SB). Faba bean (FB) increases the sustainability of crop-livestock systems through the safeguarding of soil fertility and the relatively low cost in spite of its high nutritional value due to the high protein content (25-35%). The occurrence of some anti-nutritional factors has hampered its wider utilization (Calabrò et al., 2009), however, the progress in plant breeding notably decreased their levels. Cutrignelli et al. (2008) showed that using FB as main protein source did not negatively affect animal performance; however, little data are available for buffalo. In this study the influence of replacing whole SB seed with FB in the diet of buffalo young bulls on animal performance has been evaluated.

Sixtheen males of Italian Mediterranean buffaloes equally divided into two groups, were fed after weaning, isoprotein and isoenergy diet, differing in protein source: FB tannin-free variety (Vicia faba minor L.) vs. whole seed SB (Soja hispida). Body weight and individual feed intakes were registered daily in order to calculate the feed conversion index. On the animals fasted for 24 h, the individual body dimensions were measured; on the hot carcasses, slaughtering data and carcass measurements were recorded. All data were processed to statistically test the protein sources effect.

Both groups showed a good daily weight gain; any significant differences appear between groups: 0.859 vs. 0.895 kg/d in FB and SB group. Moss et al. (1997) found no significant effects on weight gain and feed intake in young growing cattle when soybean meal was replaced by lupin seeds. Cutrignelli et al. (2008) found that the protein source (SB vs. FB) affected body weight only at 180 d in Marchigiana young bulls. All body dimensions registered were similar between groups and to those found by Infascelli et al. (1996) and Spanghero et al. (2004) in buffaloes. No difference were observed beetwen groups for body measurements in vivo and at the slaughter. The net hot dressing out mean value (53.6 %) was lower than that obtained in a previous study by Infascelli et al. (2001).

Faba bean can be used as alternative protein source to Soybean in the intensive livestock of young buffalo bulls bred for meat production as no effect on in vivo performance were observed during the experimental period. This replacement can offer decided agronomical, economical and healthy advantages.

Calabrò et al., 2009 Ita J Anim Sci, 8 (suppl. 2), 280-282. Cutrignelli et al., 2008. Ita J Anim Sci, 7, 259-270. Infascelli et al., 1996 Proc. XXXI Int Symp Zoot "Food and health: role of animal products", 155-160. Infascelli et al., 2001. Proc VI WBC, 512-518. Moss et al., 1997 Anim Feed Sci Tech, 68, 11-23. Spanghero et al., 2004. Liv Prod Sci, 91, 129-141.

Table 1. *In vivo* performance at ages and periods for the soybean and faba bean groups

Age, *d*	FB	SB	Prob.	MSE	Period, *d*	FB	SB	Prob.	MSE
	BW, *kg*					DWG, *kg*			
90	76.94	78.23	NS	209	90-150	0.616	0.618	NS	0.002
150	113.9	115.3	NS	669	150-300	0.805	0.833	NS	0.314
300	234.7	240.3	NS	3635	300-420	1.048	1.110	NS	1.538
420	360.4	373.4	NS	9858	90-420	0.859	0.895	NS	0.130
	BEG					FCI			
90	21.51	20.92	NS	26.9	90-150	4.26	4.28	NS	0.03
150	19.11	19.27	NS	10.2	150-300	5.99	5.78	NS	13.23
300	15.49	16.21	NS	13.8	300-420	7.97	7.58	NS	2.95
420	13.72	14.48	NS	13.5	90-420	7.02	6.84	NS	0.63

FB: faba bean group; SB: soybean group.
BW: body weight; DWG: daily weight gain; BEG: biological efficiency of growth (daily weight gain/body weight$^{0.75}$);
FCI: feed conversion index (feed intake/weight gain).
MSE: Mean square error. NS: not significant.

Table 2. Measurements *in vivo* and at slaughter in the soybean and faba bean groups

	FB	SB	MSE	Prob.
In vivo measurement				
Width of pelvis, *cm*	45.25	43.25	8.00	NS
Width of chest, *cm*	38.00	35.25	28.6	NS
Height at withers, *cm*	118.0	121.3	47.3	NS
Height at pelvis, *cm*	120.8	125.0	19.9	NS
Round circumference, *cm*	167.8	166.3	66.6	NS
Length of rump, *cm*	37.00	37.00	-	NS
Body length, *cm*	105.9	104.7	14.2	NS
Depth of chest, *cm*	60.25	62.00	45.8	NS
At slaughter				
Length of leg, *cm*	58.75	59.75	10.1	NS
Length of carcass, *cm*	108.8	109.3	54.8	NS
Width of leg, *cm*	35.50	34.00	6.52	NS
Depth of chest, *cm*	36.50	40.75	15.0	NS
Thickness of leg, *cm*	20.75	21.00	5.28	NS

FB: faba bean group; SB: soybean group.
MSE: mean square error. NS: not significant.

Table 3. Measurements at the dissection in the soybean and faba bean groups

	FB	SB	MSE	Prob.
Slaughter weight, *kg*	343	355	1276	NS
Net weight, *kg*	322	334	1127	NS
Hot dressing out, %	50.7	50.5	12.24	NS
Net hot dressing out, %	53.9	53.7	13.84	NS
Net cold dressing out, %	50.3	50.2	21.57	NS

FB: faba bean group; SB: soybean group.
MSE: mean square error. NS: not significant.

LINKAGE DISEQUILIBRIUM AND GENETIC DIVERSITY IN TWO SICILIAN CATTLE BREEDS ASSESSED BY BOVINE SNP CHIP

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The Modicana (MOD) and Cinisara (CIN) are two Sicilian cattle breeds farmed in extensive systems and their economic importance lies on the traditional making of two typical 'pasta filata' cheeses. The aim of this study was to explore the genetic structure and the extent of Linkage Disequilibrium (LD) of MOD and CIN cattle breeds.

A total of 144 animals were genotyped, using the Bovine SNP50K v2 BeadChip. The squared correlation coefficient between two loci (r2) was used as a measure of LD. Principal components analysis (PCA), molecular inbreeding (F) and Bayesian clustering algorithm (Pritchard et al., 2000) were used to explore the relationship between individuals and populations.

The r2 ranged from 0.018±0.026 for BTA5 to 0.106±0.199 for BTA14 in CIN, and from 0.019±0.027 for BTA2 to 0.126±0.221 for BTA14 in MOD. Differences in LD among chromosomes can be attributed to heterozygosity, genetic drift and effect of selection. The highest average value of r2 was on chromosome 14 in both breeds. Mutations in genes located on BTA14 have been found to have an effect on milk production traits. From this fact, it is possible to hypothesize that the highest value of r2 found for BTA 14 in both breeds, may be due to a selection to increase milk production traits make by farmers in an empirical way. Moreover, the value of LD in a genome determines the power of QTL detection in association mapping studies and indicates the required marker density (Meuwissen et al., 2001). The PCA showed that animals from the two breeds form non-overlapping clusters. The MOD breed clustered alone, with some individuals positioned toward the CIN breed, whereas the CIN showed two clusters. The high F values (0.68 in CIN and 0.69 in MOD) reveal a reduction of genetic variability within population. Considering a range of 1 to 6 potential clusters (K), the highest average likelihood value LnPr(G|K) with the smallest variance between replicates were obtained for K=3. The MOD is the most differentiated population with 76.8% of the individuals assigned to cluster 3, whereas the CIN animals showed a lower value of assignment with a proportion of 54.3% of the individuals assigned to cluster 1. Therefore, although animals from the two breeds clustered separately, model based clustering suggested that certain admixture has occurred and genetic links exist between both breeds.

The information generated from this study has important implications in order to maintain the genetic diversity and for future milk breeding programs. Managing of inbreeding provides a general framework to control the loss of variability avoiding or alleviating the reductions in viability and fertility; i.e., inbreeding depression.

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ANALYSIS OF THE 227 BP SINE INSERTION IN THE 5'-UNTRANSLATED REGION OF THE MYOSTATIN GENE IN DIFFERENT HORSE BREEDS

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The myostatin (MSTN) gene encodes for a protein that is well known to be a negative regulator of muscle mass in mammalian species. In previous studies we identified that two single nucleotide polymorphisms of the promoter of the MSTN gene were associated with variability of morphological traits of horse breeds (1,2). A SINE insertion (227 bp) within the 5'-untranslated region of the MSTN gene has been associated with performance of Thoroughbred racehorses and it was proposed as a good predictor of optimum racing distance (3).
The aim of this study was to analyse this SINE insertion in horses from breeds used for different purposes.

We analysed 198 horses from the following nine breeds: Haflinger (No. = 18), Italian Heavy Draught Horse (26), Italian Saddle (23), Italian Trotter (37), Lipizzan (11), Pinzgauer (13), Thoroughbred (47), Spanish Purebred (7) and Uruguayan Creole (16). Genomic DNA was extracted from hair roots. Genotyping was performed based on size determination (wild type allele= 600 bp and SINE insertion allele= 827 bp) (3). In order to identify putative transcription-factor binding sites (TFBSs) generated by the SINE insertion, sequence surrounding the SINE insertion was subjected to in silico analysis using the TFSEARCH tool (http://www.cbrc.jp/research/db/TFSEARCH.html) with a threshold score of 90.0.

Allele and genotype frequencies of the analyzed polymorphism in 9 different horse breeds are shown in Table S1. The SINE allele was found in Thoroughbred horses frequency (0.51) and, in heterozygous state, in just one horse of the Uruguayan Creole breed (0.02). The investigated polymorphism does not deviate from Hardy-Weinberg equilibrium in the genotyped Thoroughbred horses ($P>0.05$). In silico TFBSs prediction identified putative TFBSs involved in proliferation of cells (such as upstream stimulatory factor), suggesting that the SINE insertion could play a functional role.

The analyzed gene marker, that according to other publications has been associated with gallop racing performance of the Thoroughbred (3), may be population or breed specific. For this reason it might not be necessarily useful in other racing breeds such as Italian Trotter.

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Table S1. Allele and genotype frequencies of the SINE insertion in the 5'-untranslated region of the *MSTN* gene in different horse breeds

Horse breds	no. of horses	Allele frequency		Genotype frequency		
		wt allele	ins allele	wt/wt	wt/ins	ins/ins
Haflinger	18	1.00	0.00	1.00	0.00	0.00
Italian Heavy Draught Horse	26	1.00	0.00	1.00	0.00	0.00
Italian Saddle	23	1.00	0.00	1.00	0.00	0.00
Italian Trotter	37	1.00	0.00	1.00	0.00	0.00
Lipizzan	11	1.00	0.00	1.00	0.00	0.00
Pinzgauer	13	1.00	0.00	1.00	0.00	0.00
Thoroughbred	47	0.49	0.51	0.30	0.38	0.32
Spanish Purebred	7	1.00	0.00	1.00	0.00	0.00
Uruguayan Creole	16	0.98	0.02	0.94	0.06	0.00

ASSOCIATION BETWEEN THE POLYMORPHISM AT CASEIN LOCI AND MILK FATTY ACID COMPOSITION IN GIRGENTANA GOATS

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Goat polymorphism at casein genes can affect casein, fat and milk fatty (FA) acid composition. FA composition is an important trait for the goat dairy industry because of its influence on cheese yield and the organoleptic properties of dairy products (Chilliard et al., 2003). Goat milk is particularly rich in saturated fatty acids (SFA) whereas monounsaturated (MUFA) and polyunsaturated (PUFA) FA are less abundant (Fontecha et al., 2000).The aim of this work was to provide new data to better understand the influence of polymorphism at casein loci on fatty acid profile in Girgentana goat milk.

One hundred lactating Girgentana goats, homogeneous for milk production, days of lactation, body weight and diet were used. The procedure was developed using individual raw milk samples, collected in three different stages of lactation: (October, February and June) from individuals with known genotypes at casein loci. Were analyzed animals with A*A*, B*B*, A*B*, FF, A*F, B*F, A*E, EF, FN, NN and A*N genotypes (where A* indicates A, G, I, and H alleles while B* indicates B1, B2, B3, B4 and C alleles) at αs1-casein; CC, AC, A0', AC', C0', CC', and C'C' genotypes at β-casein; AA, AC, AF, CF, EF and FF genotypes at αs2-casein; and AA, AB, AD, AN, BB, BD, BN, DD and D'G genotypes at κ-casein. Milk samples were prepared following the Rose-Gottlieb's method (1996) for FA extraction. For transesterification of the lipids KOH in methanol 2 N was used and thereafter the fatty acid methyl esters (FAMEs) were analyzed with a chromatographic method (Sağdiç et al., 2003).The determination of the fatty acid profile was performed by gas chromatography SHIMADZU GC-2010 with flame ionization detector (GC-FID). The FAMEs were injected into a capillary column (Zebron ZB-WAX Plus 30m x 0:32 mm id, 0.2 mM film), identified according to the retention times and quantified by calibration curves. The results of fatty acid were expressed as g/100g total fat.Data set was analyzed using GLM procedures for repeated measure of SAS System v9.2.

For almost all the fatty acid the environmental factors flock and month of sampling showed statistically significant differences. Among all the fatty acids investigated only two showed statistically significant differences between genotypes for κ-casein, in particular C18 (stearic acid) and C18:01 (oleic acid). For αs1-, αs2- and β-casein were not found statistically significant differences.

The results showed that genetic polymorphisms at casein genes, in particular at κ-casein, have important effects on goat milk fat and FA composition, especially for MUFA and PUFA, which are potentially involved as positive factors in the health of human consumers.

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ANALYSIS OF MICROSATELLITE MARKERS IN SICILIAN GOATS BREED FOR TRACEABILITY OF GIRGENTANA TYPICAL DAIRY PRODUCTS

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The establishment of useful analytical methods able to ensure the origin of the products, including the breed used, are important in maintaining the reliability of these products in order to develop a market segment. Traceability, obtained by molecular analysis, could be a reliable proposal for the authentication and valorization of animal products. In Sicily, the three most important dairy goat breeds are Girgentana (GR), Maltese (ML) and Derivata di Siria (DS). The GR is an endangered autochthonous goat breed. Preservation of endangered breeds could be achieved by establishing economic reasons for their survival. The aim of this work was to verify the use of microsatellite markers to assess a genetic traceability system to discriminate breeds and to detect adulteration in GR dairy products.

A total of 314 individuals belonging to GR, ML, and DS goat breeds were genotyped at 24 microsatellite markers and a subset of 9 microsatellites was chosen for further analysis due to the presence of private alleles. The analyzed markers were FBC48, FBC20, CSRD247, SRCRSP5, OLADRB, SRCRSP8, OARAE54, SRCRSP24, and TGLA122. Fragments analysis of multiplex PCR was performed by capillary electrophoresis with ABI3130xl Genetic Analyzer. Allele frequency, average number of alleles, allelic richness (AR), Ho and He, PIC, and HWE for 9 loci were estimated using CERVUS 3.0.3 (Marshall et al., 1998), GENETIX (Belkhir et al., 1996), FSTAT 2.9.3.2 (Goudet, 1995) and POPGENE 1.31 (Yeh et al., 1999) software.

The results showed high variability of markers subset considering that mean PIC values was 0.6449 and average number of alleles per locus was 8.67; AR ranged from 4.585 in TGLA122 to 8.980 in OLADRB. Six microsatellite markers from subset were not in HWE probably due to heterozygote excess (Ho=0.6133).In total 4 private alleles were found in GR, 8 in DS goat breeds and 2 in ML goat breed. The most frequent private alleles were 246 bp for CSRD247 in ML (0.32) and 177 bp for SRCRSP5 in DS (0.15), respectively.

Private alleles of ML and DS goat breeds could be used for traceability of GR dairy products therefore further analysis will be performed in order to validate the possible use of these alleles on pool DNA samples extracted from mono-breed dairy products.

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RUMEN UNDEGRADABLE PROTEIN OVERFEEDING: EFFECTS ON MILK YIELD OF DAIRY COWS

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To maximize nitrogen efficiency of a dairy herd, it is important to know its protein requirements. NRC model (2001) predicts the requirements of rumen undegraded protein (RUP) for a herd in early lactation and at high production levels, (35 kg/d), but not for mid and late lactation herds (Dunlap et al., 2000). Our objective was to determine how RUP overfeeding can affect milk yield of a dairy cattle herd at mid and late lactation.

In a pre- (T0) and post- (T2) treatment period, 62 pluriparous Italian Friesian cows (parity: 3.1±1.29) at >70 days in milk (DIM) were fed a balanced diet according to NRC (2001). In a 45 days treatment period (T1), an all soybean-based ingredient (ASI) with high RUP (72% CP) was added to the diet (Table 1). RUP of T1 diet was higher than T0 and T2 (+6%) and above the average NRC requirements. Dry matter intake (DMI) and daily milk yield of the single cows was recorded for 3 consecutive days during T0, T1 and T2. Changes in DMI and milk yield were analyzed by a repeated measures mixed model using the PROC MIXED procedure of SAS with cows as random effect and DIM as covariate. Results were reported as estimate least-squares means. Significance was declared at $P \leq 0.05$.

According to previous results (Robinson at al.,1990), DMI (23.6 kg) was not influenced by RUP overfeeding. Average daily milk yield during the pre- and post-treatment periods were similar (T0: 37.4 kg/d; T2: 35.0 kg/d); during the treatment (T1), the increase of milk yield (40.2 kg) was statistically different from T2 (P=0.003).

The inclusion of an high RUP source in the diet allowed an unexpected increase and persistency of milk yield. We conclude that RUP overfeeding showed a production effect in a mid and late lactation dairy herd due to a greater N recycling back to the rumen. According to Kalscheur et al. (2006), these results may have masked the effects of a possible RDP deficiency in the diet. However, milk yield is not influenced by a lack of RDP if RUP can substitute efficiently for the lost of microbial protein.

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Table 1: Diets composition and characteristics

Diet	T0 and T2 kg/d (as fed)	T1
Corn silage	25	25
Mixed and ryegrass hay	4.5	4.5
Cracked corn	5.5	5.5
Soybean meal 44%CP	2.6	1.5
Cotton seed	1.3	1.3
Full fat soy	0.4	-
Min/Vit concentrate	2.7	2.7
ASI	-	1.5
Composition (%DM)		
DM %	55.0	55.5
Ash	8.1	8.0
Fat	4.4	4.3
NDF	34.8	34.6
Crude Protein, CP	15.5	15.6
RUP (%CP)	38	44
NEl (Mcal/kg DM)	1.56	1.57

ANALYSIS OF RUMEN FLUID SAMPLES FOR DIAGNOSIS OF METABOLIC AND INFLAMMATORY DISEASES IN CATTLE

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Previous studies indicated an active role of bovine forestomachs in the response to alimentary disorders (2), as well as to inflammatory and infectious processes in both the gastro-intestinal tract and elsewhere (1). In this respect, we showed on cows reared in an experimental station the potential of bovine forestomachs to receive, elaborate and produce signals and mediators of the innate immune response (3). The epithelial cells of the forestomachs reacted to the disturbances of the fermentation processes due to improper diets, and the inflammatory response could be sustained by infiltrating leukocytes, which secreted cytokines in the rumen liquor. The aim of this study was the evaluation of leukocytes and cytokines in the rumen liquor under field conditions as a new diagnostic approach to identify metabolic and inflammatory diseases of the ruminant species.

Rumen fluid was collected by rumenocentesis from 116 dairy cows from 11 farms about 6 hour after the main morning meal. Non-bacterial mononuclear cells (NBMC) were isolated by centrifugation on Ficoll-Hypaque (1.083 g/ml) and used for flow cytometry experiments (for discrimination of different leukocyte populations) or RNA extraction (for CD45 gene expression by Real-time PCR). The detection of IFN-gamma was performed by ELISA on rumen fluid samples. In the same samples pH and volatile fatty acids (VFA) were measured.

In the rumen fluid of clinically healthy animals (early and mid lactation) a low expression of CD45 gene, a low leukocyte infiltration - mainly consisting of B-cells (<1 % of NBMC) - and a very low concentration of any inflammatory cytokine (< 30 pg/ml) were observed. Significant divergences from this pattern (high expression of CD45 gene, different profile of leukocyte infiltration, higher levels of inflammatory cytokines) suggested a risk condition, which was related to the metabolic and immunologic profile of the animal. The analysis of the data showed that each farm had a specific profile for its animals. The ruminal pH ranged from 5.3 to 6.7 and was negatively related to the total VFA. No clear link was found between pH and leukocyte infiltration.

CONCLUSIONS: The dairy farms could be ranked according to a risk score using the mentioned inflammatory markers from rumen fluids. These markers could integrate the usual, consolidated information (e.g. rumen pH and VFA, milk cell counts, drug consumption). The veterinary practitioner could take advantage of this additional diagnostic approach for a more comprehensive assessment of the metabolic diseases and of the inflammatory conditions of dairy cattle.
ACKNOWLEDGEMENTS: this study was partially funded by Nuova Padana Mangimi (Vigorovea di S. Angelo di Piove, Padua, Italy)

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DIET EFFECT ON POSTPRANDIAL GLYCEMIC RESPONSE IN CAT

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In carnivores starch digestibility is highly variable and affected by several factors. Starch is the main nutrient, which affect postprandial glucose responses in dog and cat (de Olivera et al., 2008). Other dietary factors that influence these responses are protein/starch, dietary fiber and unsaturated fatty acids (Carciofi et al., 2008). The aim of this study was to evaluate the dietary effects on glycemic profile of healthy adult cats.

Six European adult cats in health conditions (weight 4.6 ± 0.3 kg, BCS 5.7 ± 0.5, age 3.5 ± 0.2 years) were utilized. Throughout the entire experimental period cats live with during the trial with their adoptive families. Four commercial dry extruted diets, differing in composition and nutritional characteristics were used: diet VHP/LS (free from cereals and characterized by low starch and very high protein levels), HP/MS (characterized by moderate starch level from oats and spelt and high protein content) and two traditional diets (MP/HS1 and MP/HS2, characterized by high contents of starch from rice and corn and moderate protein content). All diets were administered (100 kcal ME/kg0.67/d) for 30 days (10 adaptation + 20 trial) according a cross over design (6 cats x 4 diets x 30d). At the end of each period, fasted cats were weighed and blood samples were collected to measure fructosamine levels. Serum glucose levels was determined 12 times during 24 h. All data were statistically analysed to evaluate diet effect (Proc GLM of SAS, 2000).

During the experimental period there were no statistically significant changes for body weight and food intake. For these reasons the diets could be considered equal for energy contents and different for protein and starch contents (CP: 28; 28; 42; 33 % DM starch: 17; 26; 34 and 35 % DM for diets MP/HS1, MP/HS2, VHP/LS and HP/MS). Significant differences were observed for the glycemic and fructosamine values (Mean glyc: 74.67; 74.03; 67.64; 62.83 mg/dl, P <0.05; Peak: 82.57; 83.4; 72.86; 71.33 mg/dl, P <0.01 for MP/HS1, MP/HS2, VHP/LS and HP/MS); Fr: 347; 333; 318, 246 mmol/l, P <0.05 for diets) any significant differences were observed between the diets MP/HS1 and 2, while diet VHP/LS showed for all parameters values lower than HP/MS. In the graph are described the mean glycemic response with the diets.

The use of diet with high Protein/Starch can modulate the postprandial glycemic response also with long-term effects on glucose metabolism, as evidenced by the significant differences recorded for fructosamine levels. Particularly interesting is the observation that diet VHP/LS, characterized by very high protein levels of protein and low starch concentration form patties allow to obtain the more modulate glycemic response in cat.

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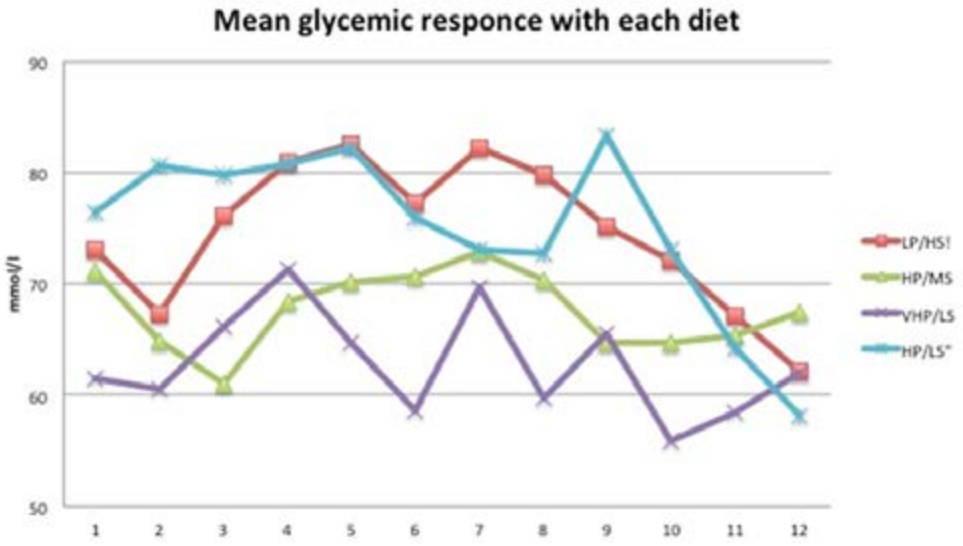
Mean glycemic responce with each diet
mmol/l
90
80
70
60
50
1
2
3
4
5
6
7
8
9
10
11
12
LP/HS!
HP/MS
VHP/LS
HP/LS"

THE EFFECTS OF SOYBEAN PRESS CAKE ON PARMA HAM QUALITY

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Despite of a lack of specific recent literature, press cakes (mechanical extraction) are not presently allowed in PDO (Protected Designation of Origin)hams production (1) due to possible negative effects on fat stability during seasoning tied to their high linoleic acid content. On the other hand, press cakes probably represent the more feasible way to fulfill the amino acidic requirements of pigs raised according to the Organic Method (2). The present trial was aimed to give more insights on the possibility of totally replacing soybean solvent-extracted meal with soybean cake in the diets of heavy pigs intended for Parma Ham production.

Sixty crossbred pigs (initial BW 26 kg) were offered two isoenergetic and isoaminoacidic diets: a control group was fed a diet containing soybean meal (SBM), treatment group received a diet containing soybean cake (SBC). Pigs were slaughtered at 160 kg BW.
Right thighs were followed during the whole dry-curing process (18 months)to calculate weight losses for each productive step. 32 hams (16 for each group) were sensorially evaluated by a panel of five trained experts. Samples of Biceps femoris muscle were analyzed for moisture, crude protein, NaCl and proteolysis index. Fatty acids composition, peroxides and TBARS were assessed in subcutaneous fat.
The experimental data obtained were submitted to analysis of variance with the diet assumed as the main effect.

No significant differences ($P>0.05$) were observed during the whole dry-curing period with respect to ham weight losses. Sensory analysis didn't reveal any significant difference between the experimental groups with respect both to the lean fraction (humidity, texture, marbling) and the subcutaneous fat (thickness and texture). Chemical analyses showed a significant ($P<0.05$) reduction in the proteolysis index in the group receiving the SBC diet. However, all chemical parameters fell within the range imposed by Parma Ham (1). The acidic composition of the subcutaneous fat from the dry-cured hams showed a significant ($P<0.01$) increase in PUFA and a reduction in SFA and MUFA in the SBC group compared with the SBM group. Such a modification reflects the acidic composition of the diets and that of fat from the raw thighs. However, the overall oxidative stability (peroxides and TBARS) didn't differ significantly between the groups (Table 1).

Our results suggest that the total replacement of soybean meal (solvent-extracted) by soybean cake (mechanically-extracted)does not modify either the main chemical and sensorial traits or the oxidative stability of cured hams. Therefore, such a replacement appears to be a feasible means to achieve entirely organic diets for heavy pigs without compromising PDO hams quality.

1) Consortium for Parma Ham (1992) http://www.prosciuttodiparma.com/pdf/en_UK/specifications.pdf. 2) EC (2007) Council Regulation 2007/834/EC OJEC L 189, 1–23.

Table 1 –Ham qualitative traits, fatty acid composition and oxidative status of subcutaneous fat

group	SBM	SBC	RMSE
Samples (n)	16	16	-
Moisture (%)	60.82	60.91	1.64
Proteolisys index (%)	26.68^{a}	25.10^{b}	1.91
Salt (%)	6.61	6.69	0.81
C 16:0 (%)	21.97^{A}	20.75^{B}	0.72
C 16:1 (%)	2.43^{A}	1.99^{B}	0.36
C 18:1 (%)	45.58	44.01	2.36
C 18:2 (%)	10.64^{B}	13.72^{A}	2.15
C 18:3 (%)	0.63^{B}	0.95^{A}	0.14
SFA (%)	34.56^{A}	33.09^{B}	0.96
MUFA (%)	53.30^{A}	51.08^{B}	2.18
PUFA (%)	12.15^{B}	15.83^{A}	2.38
Peroxides ($meqO_2/kg$)	9.24	10.67	3.20
TBARS (MDA mg/kg)	1.36	1.33	0.55

a,b P<0.05; A,B P<0.01

APPLICATION OF MICROSCOPIC METHODS AND COMPUTER IMAGE ANALYSIS FOR THE IDENTIFICATION OF BOVINE AND SWINE MEAT AND BONE MEALS

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The reintroduction of non-ruminant PAP in aquafeed was voted and adopted on 18th July 2012 at the European Commission level. To that purpose, PCR was added to the microscopic one, as official method for detecting the species origin of PAP in a new version of annex VI of regulation 152/2009 (Reg 51/2013). Nevertheless none of the method is able on its own to fit all requirements for the accurate identification of prohibited ingredients of animal origin (e.g. differentiate between authorized and prohibited ingredients), leading to propose a combinatory approach in which all methods can be used, implemented, and eventually merged. Accordingly the aim of this work was to combine the official analytical method with the image analysis measurements for the identification of bovine and swine meat and bone meals.

For this study, authentic samples of controlled origin containing bovine (BOV) or swine (SUS) meat and bone meals were analyzed by the microscopic method, according to Annex VI of Regulation 152/2009. Sediment fractions of each sample were observed with a compound microscope at X40. Three hundred sixty two bone fragment lacunae images were recorded and processed through an image analysis software. Accordingly 30 geometric variables were obtained, as previously described by Campagnoli (2009). Data obtained were analyzed by ANOVA (GLM procedure) and by PROC BOXPLOT procedure of SAS statistic software 9.3. In order to show the variability of the most discriminant variables we have performed a graphic test (box-plot).

Results obtained in the present study indicated that not only most of variables (15) measured were significantly (<.001) different between bovine and swine, but also that 2/3 of the same variables were bigger in bovine than in swine. These information however, seem to be not so effective in practice since features of bovine and swine material overlap as has been evidenced by the graphic test (box-plot) for mean and median comparisons.

From this study it can be concluded that the microscopic method even when improved is not able to fit all requirements for the accurate identification of prohibited ingredients from animal origin, and therefore a combinatory approach should be recommended. However, present study is based on a limited number of samples and additional observations/studies are needed, in order to get an in-depth analysis.
[This study has been done in the frame of Progetto di Ricerca Corrente 2010, n° id. IZSPLV 12/10/RC dal titolo "Identificazione di specie delle proteine animali trasformate nei mangimi: sviluppo e confronto di tecniche microscopiche e immunoistochimiche”]

Campagnoli A., Paltanin C., Savoini G., Baldi A., Pinotti L., (2009). Combining microscopic methods and computer image analysis for lacunae morpho-metric measurements in poultry and mammal by-products characterization. Biotechnol. Agron. Soc. Environ. 13(S) : 25-27. Ed. by Les presses agronomiques de Gembloux Belgium.

PRELIMINARY INVESTIGATION ON THE USE OF THERMAL IMAGING FOR EVALUATING VULVAR SKIN TEMPERATURE CHANGES IN SOW

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Infrared Thermography is a technique based on the detection of thermal energy emitted by a body and converted into an electronic signal, which in turn is processed by software to produce digital image. Infrared Thermography does not require any contact and is therefore a completely non-invasive technique for assessing the physiological state of animals and recording measurements on subjects difficult to reach or to approach. Two studies on gilts and sows (Scolari et al., 2011; Simoes, 2012) have proved that vulvar surface temperature changes significantly in pre-ovulatory phase, with peaks and drops which occur few hours before ovulation. In order to check the practical application of thermography in artificial insemination (AI) management, a preliminary study was performed for assessing the trend of vulvar area temperature on a group of sows after weaning.

Infrared images of vulvar area were recorded for six consecutive days at 7 a.m. on 24 primiparous and pluriparous sows of Hermitage commercial hybrid, chosen irrespectivetly of age and body condition. The estrus research and/or stimulation with boar were carried out two time a day from two days before the beginning of measurements. On the total of sows, 15 were inseminated after 4 days from weaning and 9 after 5 days, respectively. Thermal images were recorded at a fixed distance of m 1.0 with a portable camera FLIR E40bx (FLIR System Srl, Milan), setting the emissivity equal to 0.98 and calibrated the camera with environmental temperature and relative humidity. Each thermal image was analyzed by Flir Tools.Ink software (FLIR System Srl, Milan). Maximum temperature of area around the vulva was recorded and successively processed by ANOVA for repeated measures.

Graphic 1 shows the variation of the maximum temperature in the examined area. There was a significant increase ($P<0,05$) between the first and second day , the latter corresponding to the estrous, followed by a fast decline, corresponding to the start of ovulatory phase. It is evident that a single thermal image of vulvar area recorded once a day by infrared thermo-camera, is sufficient to detect heat and the following onset of ovulatory phase, suggesting the potential of infrared thermography for the detection of best moment for AI.

Infrared thermography appears a promising tool for monitoring estrous and successive ovulatory phase supporting the farmer to organize the AI management of sows.

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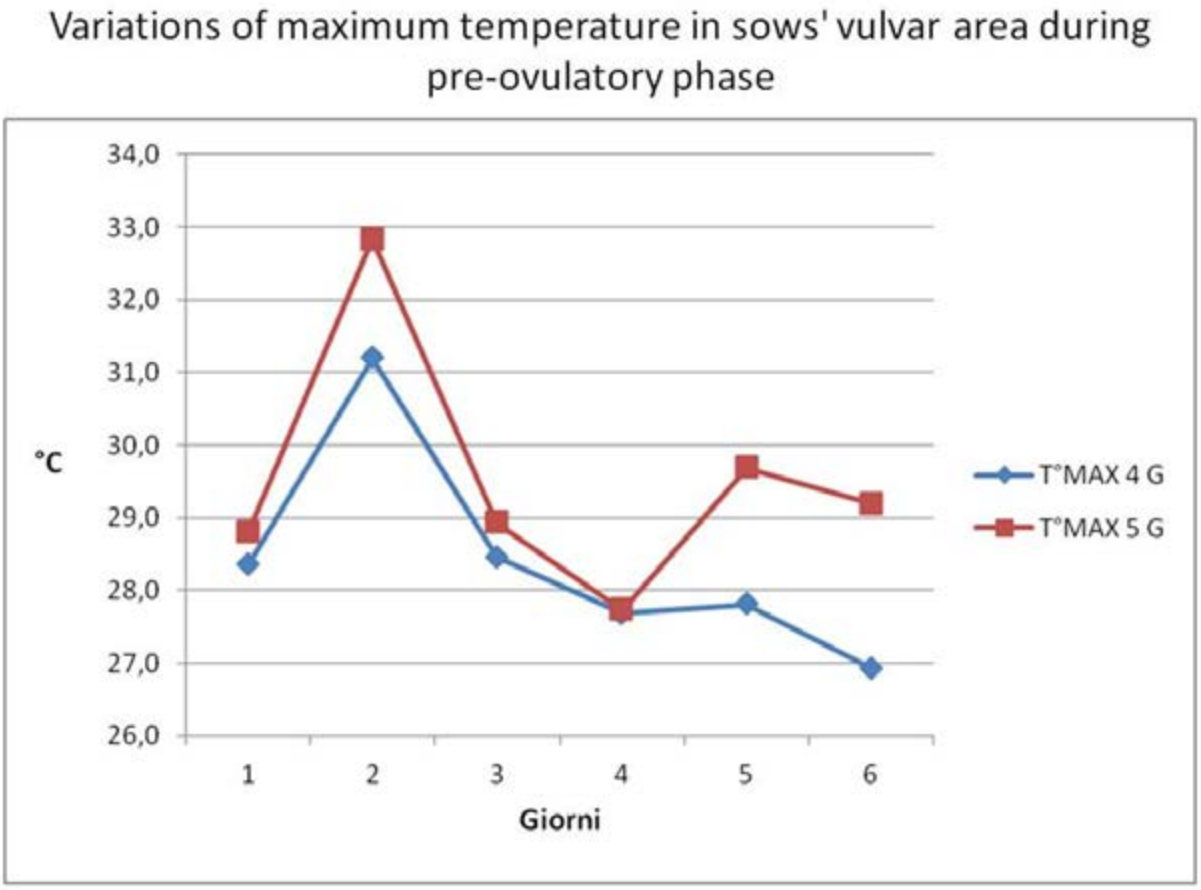
Simoes V. J. G. (2012).Variation in the vulvar temperature of sows as determined by infrared thermography and its relation to ovulation. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa


Variations of maximum temperature in sows' vulvar area during pre-ovulatory phase
34,0
33,0
32,0
31,0
30,0
29,0
28,0
27,0
26,0
°C
1
2
3
4
5
6
Giorni
T°MAX 4 G
T°MAX 5 G

CACIOCAVALLO PALERMITANO CHEESE: CHARACTERIZATION OF LACTIC ACID BACTERIA (LAB)

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Caciocavallo Palermitano is a typical “pasta-filata” cheese produced in Western Sicily using raw milk from autochthonous cow and according to traditional cheese-making technology based on the use of wooden tools. The aim of the study was to investigate lactic acid bacteria (LAB) variability and to evaluate the influence of the wooden dairy tools on the microbiological characteristics of the cheese.

The study was conducted on 42 Caciocavallo Palermitano cheeses of different ages, produced in 30 dairy farms. The samples of cheese were examined for total microbial count (TMC) at 30°C and for LAB using Rapporti ISTISAN 08/36. In order to explore the bacterial ecosystem of cheeses, molecular fingerprinting of the bacterial isolates was conducted in cheeses coming from different dairy farms at different periods of ripening and native microflora biofilms from the wooden vats called "tina" were investigated in six dairy farms.Genotypic identification of the LAB isolates was carried out by 16S/23S rRNA intergenic spacer region (ITS) gene sequencing. PCR reactions was performed as described by White et al. (1990). DNA sequences were compared by a BLAST search in GenBank/EMBL/DDBJ database.

TMC values were ranged from 103 to 108 cfu/g and decreased from the third month of the ripening. The fresh cheeses (< 1 month ripening) showed high concentrations of LAB between 106 and 107 cfu/g. The rod-shaped LAB maintained at a constant concentration of 106-107 cfu/g in all the samples. The coccus-shaped microflora, on the contrary, tended to decrease until it reached concentrations of 103-106 cfu/g in the cheeses with an higher ripening. LAB isolates (152) were variously represented by Pediococcus, Enterococcoccus, Streptococcus e Leuconostoc. Lactobacillus casei, Lactobacillus rhamnosus, Pediococcus acidilactici, Pediococcus pentosaceus and Enterococcus faecium dominated the microbial community.
Isolated colonies from the wooden vat surface showed predominance of mesophilic cocci (105-106 cfu/cm2), thermophilic cocci (104-106 cfu/cm2) and mesophilic lactobacilli (104-106 cfu/ cm2). Streptococcus thermophylus e Lactobacillus casei dominated the microbial community of the wooden vats.

The study revealed the presence of a viable lactic flora in the cheese, consisting predominantly of lactobacilli. These microorganisms come from raw milk, are selected during the cheese-making processes in relation to the use of wooden tools and give the cheeses typical characteristics.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York, 315-322.

VALIDATION OF A HIGH LIQUID CHROMATOGRAPHY METHOD (HPLC) FOR SIMULTANEOUS QUANTIFICATION OF SKATOLE AND INDOLE IN PORCINE FAT

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Boar taint is the offensive odor due to excessive accumulation of skatole, indole and androstenone that may lead to an unpleasant experience during the consumption of meat or meat products from male pigs; the bad smell increases when meat is heated or during cooking (1). To relate boar taint and molecules concentration, a fast identification and quantification of skatole and indole, via high-pressure liquid chromatography, coupled with a fluorimetric detector (HPLC-FD), has been set up and validated.

The extraction of two out of three target molecules (namely: skatole, indole), both with the internal standard (2-methylindole) from subcutaneous fat (0.5±0.05g) taken at slaughterhouse from control female pigs, was performed, after sample liquefaction, by the addition of pure methanol. Separation of the organic solvent was obtained by cooling and centrifuging (2). For the method validation, linearity, recovery, accuracy and sensitivity were evaluated, further to the detection (LOD) and the quantification (LOQ) limits.

For both molecules the correlation coefficient (R2) of the standards calibration curves was always ≥ 0,99, confirming the good linearity, while recoveries from fat matrix were always ≥ 96%. The intra-and inter-assay precision of the method, expressed as relative standard deviation (RDS%), for each analyte, were always < 10%; LOD and LOQ, were respectively 1.562 ng/g and 2.312 ng/g for both compounds. The method was then applied to subcutaneous fat samples taken at slaughterhouse by different subjects (sows, entire and castrated male; n° 28) allowing the identification and quantification of skatole and indole, in the range of 174.43 ± 258.14 ng/g; and 124.5 ± 99.33 ng/g respectively, thus confirming the robustness of the method.

Thanks to the high sensitivity and precision of the method, the concentrations detected were far lower than those perceived by human nose. We hope that the reduced sample amount, used during the validation method, could be suitable for high-throughput routine analyses of samples collected from live breeding candidates by a subcutaneous fat biopsy device, identifying low-risk boars in a breeding program as reported by Baes(3).

1) Bekaert K. M. et al.,(2012) Journal of Chromat A, 1239: 49-55. 2) Pauly C. et al.,(2011) Animal, 5(3): 378-386. 3) Baes C. et al.,(2013) Animal, 7(5): 714-720.

STUDY OF MASTITIS IN DAIRY SHEEP USING SURVIVAL ANALYSIS

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Mastitis is the most prevalent disease present in livestock species leading to economic loss. In dairy sheep, it caused mainly from bacterial infections. The aim of this work was to investigate the risk of having mastitis in Valle del Belice dairy ewes during the first lactation, due to environmental or contagious pathogens, using a survival analysis approach.

All test-day records from primiparous ewes were collected from five flocks. All test-day were grouped in two data sets, one with mastitis due to environmental pathogens (ENV) and the other with mastitis due to contagious pathogens (CON). In this analysis the follow up period of a ewe was the lactation, consequently all the record began with lambing. Ewes without infection at the end of the first lactation were treated as right censored. The analysis was performed with the Survival kit v.6.1 (Ducrocq et al., 2010). Cox model was used: h(t; x_m,z_m)=h_0 (t)exp{x_m^' (t)┤ β+z_m^i u where: h_0 (t) is the baseline hazard; β contains the time-independent covariates: litter size (LS, 2 classes), age at first lambing (AFL, 4 classes); and time-dependent covariates: milk production within flock (MK, 3 classes); somatic cell count within flock (SCC, 3 classes); EP: environmental pathogens, that was only considered when analyzing contagious pathogens (2 classes) and CP: contagious pathogens, that was considered only when analyzing environmental pathogens (2 classes); u contains the time-independent random effect of FYS: flock-year-season (46 classes).

For ENV and CON about 48% and 85% of the records were right censored, respectively. Chi-square test approximations based on likelihood ratio test were calculated for all the effects in the model. For the ENV, the time-dependent fixed effects: MK, SCC and CP were significant. A higher risk of being infected (hazard ratio, HR, 1.41) was observed for ewes with low MK compared to reference class (i.e. MK=2). Higher HRs were found for animals that had a medium and high level of SCC (1.80 and 2.23, respectively) compared to the reference class of low level of SCC. A lower HR was observed for animals that have been infected with contagious pathogens (0.08). For CON, SCC and EP were significant:. A higher HR was found for animals that had a medium (1.79) and high (3.96) level of SCC compared to the reference class of low level of SCC. A lower HR was observed for animals that have been infected with environmental pathogens (0.09).

Results suggest that selection for decreased SCC may be effective to reduce mastitis incidence and the breeding goal should favor ewes with lowest observed SCC.

Ducrocq, V., Solkner, J. and Meszaros G. (2010). Survival Kit v6 a Software Package for Survival Analysis. In: 9th World Cong. Genet. Appl. Livest. Prod., Leipzig, Germany.

LISTERIA MONOCYTOGENES IN BULK TANK MILK: EVALUATION OF ITS BEHAVIOR DURING THE CHEESE MAKING AND PREDICTIVE MODEL VALIDATION.

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In 2012 bulk tank milk (BTM) from 258 dairy herds in province of Brescia - Lombardy Region - was examined for the presence of foodborne pathogens. In particular, Listeria monocytogenes was detected by Real-Time PCR with 2.7% (7/258) BTM positive samples, 1.16% (3/258) of which was confirmed by microbiological test.
The follow up performed on all the lactating cows of the positive farms always showed L. monocytogenes from a quarter milk sample of a single animal as source of whole milk contamination. One carrier cow, shedding 103cfu/ml, was able to contaminate the bulk tank milk.
Aim of this study was to evaluate the behavior of indigenous L. monocytogenes in cheese (60 days ripening) made from natural contaminated milk and to compare the observed result with the result generated by a predictive model, that considers the cheese environment.

In order to assess the behavior of L. monocytogenes during the cheese making and ripening, two batches of contaminated milk (25 L milk/batch) were used. Milk, curd and cheese samples were used to evaluate the pathogen and LAB (Lactic Acid Bacteria) levels, the pH and aw values. The temperature during the cheese making was registered by a data logger. All analyses were carried out in triplicate. To predict the behavior of the pathogen during the manufacture of cheese, was used a mathematical model (Le Marc et al. 2002), that considers the effect of temperature, pH and the starter culture on the growth of L. monocytogenes.

During the manufacture of cheese, the LAB increased from ca. 6.7 to 9 log cfu/g in the first four days. This increase in LAB levels generates a slight acidification of the cheese. An increase in the concentration of L. monocytogenes level (from 3.5 to 5.7 log cfu/g) was observed during the first days of ripening. The growth of pathogen then stops until the end of the ripening period. The results shown that the LAB are able to induce an early stationary state in L. monocytogenes: the growth of pathogens is inhibited when the LAB have reached a critical density (Jameson effect). The mathematical model confirms the pathogen behavior, indicating that the use of predictive microbiology can be a valuable tool for assessing the behavior of pathogen in food in LAB presence. The results show even that an indigenous pathogen is not sensible to the cheese environment; it is able to growth two logarithms during the cheese making, as described by the mathematical model. Moreover, the result suggest that, the initial L.monocytogenes concentration in milk need to be less than 1cfu/ml to produce raw milk cheese ripened for 60 days or less.

The correct risk analysis has to consider the low incidence of L.monocytogenes in raw milk, but is necessary to take in account the possibility that the BTM could be contaminated.

Le Marc, Huchet, Bourgeois, Guyonnet, Mafart, Thuault (2002)Int J Food Microbiol, 219-237

EMPIRICAL PREDICTIVE MODEL TO DESCRIBE THE BEHAVIOUR OF LISTERIA MONOCYTOGENES DURING THE SHELF LIFE OF BLUE-VEINED CHEESE

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In the online database, Rapid Alert System for Food and Feed (RASFF), created by the European Commission, it is published that in the last ten years almost 40 vs 58 notifications of Listeria monocytogenes in dairy products belong to Italian blue veined cheese. In fact, among dairy products, the blue veined cheese, is known to be the most frequently contaminated. The eco-system of this cheese changes strongly during the aging and ripening. Mainly, the pH increases due to the proteolytic enzymes effect. The increase of pH during ripening enhances the possibilities of survival and growth of L. monocytogenes.
The purpose of this work was to investigate the behaviour of L. monocytogenes in blue-veined cheese during the shelf life using the predictive microbiology tools. Linking the temperature to the growth rate of L. monocytogenes allows the development of a simple decision aid tool in which for each time/ temperature profile given is possible to see the logcell concentration increase.

Two L. monocytogenes strains, a registered and a wild strain type, were used in this experiment.
Three batches of blue veined cheese were considered. Each batch was divided in three parts; control, inoculated with registered strain and wild strain. The control test units (10g of cheese each) were inoculated with sterile physiological solution, consecutively the other test units were inoculated with the registered strain or wild strain respectively (1/100v/v). The final pathogen concentration in cheese was about 1000cfu/g. The test units were then incubated at 6, 10, 12 °C. For the enumeration of L. monocytogenes was used an accredited method (ISO, 1998).
The predictive model was carried out in two steps. First, for each growth curve, the specific growth rate (µmax) was calculated using DMfit Excel® add in, based on Baranyi and Roberts model (1994). Then, the secondary model was obtained by regressing the natural logarithm of observed µmax to the respective temperature. To obtain predictions for the bacterial concentration during time-dependent temperature profiles, the dynamic model was solved numerically using the second order Runge–Kutta method in an Excel® spread-sheet. Bias and discrepancy between model predictions and observations were estimated as reported by Baranyi et al. (1999).

In blue veined cheese, the µmax of L. monocytogenes varied from 0.015 h-1 at 6°C to 0.08 h-1 at 12°C with a µmax standard error (SE) of fit between 0.78 and 0.93. The adjusted R2 value for the secondary model was 0.71 with a SE of 0.27. The discrepancy between the predicted and observed data was acceptable. The model was used to develop an Excel® add in decision aid, useful for FBOs to evaluate the safety of their product, also in case of temperature abuse.

The software will be published on www.ars-alimentaria.it, an Italian food safety database, funded by Italian Health Ministry.

Baranyi and Roberts 1994 Int. J. Food Microbiol.
Baranyi Pin and Ross 1999 Int. J. Food Microbiol.

RISK ASSESSMENT AND QUALITY ASSURANCE IN AN ITALIAN FEED INDUSTRY

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The recent feed and food-related safety crises were important impulses to enhance the quality programme, which has resulted in the integration of European legislation, in the involvement of risk analysis in entire feed production chain by integrating the Hazard Analysis and Critical Control Point (HACCP) principles and in the extension of the quality assurance to all suppliers of feed ingredients. (Johan den Hartog, 2003; Pagliarulo et al. 2007).
The aim of this study is to evaluate the efficiency of the quality assurance system in an Italian feed industry assuring to produce and supply safe animal feeds.

The risk assessment and quality assurance system (based on Reg. CE 183/05, HACCP principles and EN ISO 9001:2008) of a feed industry were examined and implemented. The applied HACCP principles were: conduct a Hazard Analysis (HA); identify Critical Control Points (CCPs); establish Critical Limits (CLs); establish CCP monitoring requirements; establish Corrective Actions (CA); establish verification procedures; establish record-keeping procedures.
Furthermore, the risk analysis steps were: identification of product and processing; identification of critical steps; identification of possible hazards (chemical, physical or microbiological); determination of risk level (probability that a hazard will occur multiplied by its severity); determination of the relevant risks and critical points in the production process (FAO, 2010).

The following critical points were recognized: receipt of raw materials, dosing and weighing ingredients, cross-contamination during processing. Only dosing and weighing (both automatic and manual procedures) have been recognized as a CCP since every action is registered by the processing control software, connected to weighing hoppers and balances. The analysis performed on raw materials and the accurate selection of any supplier according to internal requirements guarantee the consumption of safe ingredients. The cross-contamination control procedure avoids effectively contamination of different batches. A computerized system allows to realize accurate product traceability.

Risk-based programmes and HACCP have proved successful in achieving hazard control to the extent required for consumer protection (Verstraete, 2013). Anyway constant monitoring of the system is needed to ensure its success.

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EFFECT OF STARVATION ON WELFARE PARAMETERS IN AMERICAN LOBSTER (HOMARUS AMERICANUS)

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The American lobster (Homarus americanus) is of great commercial importance and has a high demand in the international market. Lobsters are traditionally marketed live and stored for several weeks in non-feeding conditions and the current legislation does not limit the crustacean storage time. Research has highlighted the negative effects on the crustacean welfare of non-optimal management procedures during transport and storage (Elwood et al., 2009; Lorenzon et al., 2008). The aim of this study was to evaluate the effect of food deprivation on lobster welfare parameters, including muscle myofibrillar protein damage.

This study was performed in the aquaria of the Food Science Department, University of Udine. During the experiment, water parameters were: temperature, 8.1 ± 0.70 °C; O2, 7.0 ± 0.26 mg/l; pH, 7.5; N-NH4, 1.2 ± 0.46 mg/l; N-NO2, 2.1 ± 1.48 mg/l; N-NO3,<5 mg/l. 42 adult Homarus americanus males were starved in four recirculating tanks for four weeks and weekly weighed. A daily photoperiod of 12 h was maintained during all the experiment. Hemolymph samples were withdrawn from the pericardial sinus of each animal at the beginning of the experiment and after every week. Samples were tested for glycemia, total protein content and total haemocyte count (THC). Abdominal muscle samples were extracted for proteins in sodium dodecyl sulfate (SDS) solution containing 100 mM dithiotreitol (DTT). Extracts were analyzed using one-dimensional denaturing electrophoresis (SDS-PAGE) followed by Western blotting with anti-actin antibody.

Glucose, total protein and THC in the hemolymph of lobsters were rather constant during the experiment, whereas an increase of the crustacean weight was observed, possibly related to restoration of water imbalance due to transportation. By the end of the experiment, the electrophoretic analysis revealed a marked degradation of myofibrillar proteins. A number of fragments, possibly from myosin, were evident in the range between 220 and 50 kDa. A proteolytic product at 37 kDa recognized by the anti-actin antibody, was also detected.

Crustaceans are able to display a wide range of adaptation strategies to survive and grow. The overall knowledge of these complex biological systems could be of importance in order to identify useful welfare indicators. In this study, muscle degradation appears to be a sensitive indicator to monitor response to starvation. Both calcium-dependent (calpains) and ubiquitin (Ub)/proteasome-dependent proteolytic systems, which contribute to enhanced turnover of myofibrillar proteind during programmed atrophy (Mykles, 1997), may be responsible of protein turnover in our non-feeding conditions.

1) Elwood R.W., Barr S., Patterson L. 2009. Appl. Anim. Behav. Sci. 118: 128-136; 2) Lorenzon S., Giulianini P.G., Libralato S., Martinis M., Ferrero E.A. 2008. Aquaculture, 278: 156-163; 3) Mykles, D.L. 1997. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 117: 367-378.

EVALUATION OF THE MICROBIAL POLLUTION IN A FARM FOR CONVENTIONAL RABBITS USING AN AIR SAMPLER

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We have developed a working protocol in order to evaluate the air contamination in different kinds of rabbitries for breeding rabbit used for experimental procedures using an easy instrument for sampling, the SAS® System (PBI International, Italy) and plates filled with different types of cultural media. [1-2]

The two trials have been performed in a rabbit farm located in the NW of Italy. White New Zealand rabbits (NZR) were housed in two different rooms of the same building, with forced ventilation, one for does and one for fatteners. For air sampling we have used the SAS® System that is an impaction method that allows to enumerate the number of microorganisms directly in elevated convex plates filled with cultural media. A known volume of air is thrown onto a Surfair plate; then the sample is incubated depending on the microorganisms we want to isolate. The results are expressed as CFU (Colony Forming Unit)/m3.

During the first trial developed in the rabbit farm, the bacterial charge we have observed was always above 50 CFU/m3 of aspired air; Micrococcus luteus and Staphylococcus spp. were the most isolated microorganisms in both kind of samples.
For fungi, 100% of plates were positive for environmental fungi and yeasts such as Aspergillus spp., Penicillium spp., Alternaria spp. and Rhodotorula rubra but their number remained under 50 CFU/m3. The percentage of dermatophytes isolated was between 50 and 70 and the only species identified was Trichophyton mentagrophytes as in opened plates. [3]
During the second trial, the microbiological results showed a total bacterial charge between 50 and 100 CFU/m3 air flow, using the SAS® System. On the contrary, the total fungal charge (environmental fungi and dermathophytes), keeps below 50 CFU/m3. These values could be considered low (50-100 CFU/m3) and very low (<50 CFU/m3) with regards to the environmental risk according to the parameters supplied by the SAS System's producer. The isolated microorganisms were Bacillus (100%), Alternaria (11,09%), Aspergillus niger (36,12%) and Microsporum gypseum (41,67%). None of the bacteria/fungi that we have isolated was strictly pathogens.

So, the method we have applied for the evaluation of the microbial air quality allows us to obtain good and reproducible qualitative and quantitative results while opened plates allow only a qualitative results. Moreover, the SAS® System is portable and noiseless and it is optimal for sampling air in the nests and in cages or in a facility room.

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EFFECT OF REARING SYSTEM ON CARCASS TRAITS AND MEAT QUALITY OF RABBIT

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The factors influencing the productivity in farm animals are multiple and linked to each other.
The breeding environment as a prior relevance because it recreates natural conditions animals should live in. The aim of this research is to analyze and evaluate the effects of different types of housing, cages and diet on productivity, well-being and quality of rabbit meat.

The experiment was managed at a commercial rabbitry in the province of Padua. The testing involved a complete production cycle and included a total of 432 commercial hybrids Hyla. The experiment was divided into 2 parts: "in vivo", recording zootechnical performances and "post-mortem", analyzing their carcasses. It was considered two different environments stabling and two different diets. In this seat we will deal the results of the analysis of the carcass [1].

The daily weight gains and the final weights were greater in rabbits placed indoor, in conventional cages with standard diet. [1] The model of cage did bring significant differences between subjects in conventional cages that are heavier at slaughter than those reared in modified cages (3.1 vs. 2.8 kg).
The parameter for the average weight/head showed that the placement of the cage inside or outside did not affect this value (2.9 kg). The position of the cages did not significantly alter the composition of the carcass, even if it's been a trend of subjects reared outdoors to present heavier carcasses and organs.
The cage has changed significantly worsened all those features. The rabbits housed in conventional cages showed higher slaughter weight (3.1 kg, $P < 0.05$), due to a more limited movement and a free access to the feeder. [2-3] They also showed significantly higher weights for the whole carcass for the thighs, for some internal organs and the separable fat.
The diet did not affect the pH of the carcass after 24 h.
.

These observations considered, it is clear that the type of structures adopted is one of the most important aspects to consider to ensure adequate housing conditions for animals and to improve their welfare. [3] Although it is clear that the issue of animal welfare in rabbits should be faced with a multidisciplinary approach that does not underestimate other aspects of the management of breeding, such as the introduction of appropriate biosecurity measures, the pursuit of improvement projects and genetic health of breeding and, then, proper management of animals and their production cycles.

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THE AUTOCHTHONOUS AGEROLESE BREED: GENETIC STRUCTURE AT CSN1S1 AND CSN3 LOCI

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This work arises as targets the genetic characterization at CSN1S1 and CSN3 loci, using techniques of molecular biology, of the agerolese cattle, an autochthonous genetic type reared in Campania, which is at risk of extinction.In particular, we proceeded to the identification of the CSN1S1 G and CSN3 B allele carriers, whose choice was dictated by the documented importance of their products (as1-CnG and k-CnB).The molecular event that characterizes the CSN1S1 G allele is an insertion of a truncated retroposon-like element (LINE) of 371 bp realized between nucleotides 58 and 59 of 19th exon. Consequently, the 19th exon of bovine CSN1S1 G allele appears to be 756 bp long instead of 385 bp[1].The cause of a reduced efficiency of protein synthesis has been hypothesized to be an interaction between the LINE nucleotide sequence and the poly(A) sequence of the mature transcript, with consequent reduction of mRNA stability and its rapid degradation.The mutation responsible for the CSN3 B allele is a nucleotide substitution (GATAsp-GCTAla) at nucleotide 416 of 4th exon. This mutation is located relatively close to several glycosylation sites and probably affects the structure of the protein and glycosylation patterns[2]. It was reported that the CSN1S1 G allele is responsible for a considerable reduction of the content of as1 casein that involves a lower coagulation time and curd firmness of milk compared to that produced by cows bearers of the remaining alleles[3].Similarly, it was shown that the k-CnB variant influences the processes of cheesemaking, giving the milk a better attitude to cheese, with lower clotting times and firming, greater consistency of the curd and higher yield. From the milk obtained by the CSN3 BB cows it can get a higher yield in "Parmigiano Reggiano" equal to ~10%[4]

The genomic DNA was extracted from 79 individual samples of bovine blood reared in different farms located in Campania. For the genotyping of CSN1S1 G allele carriers was applied a PCR protocol[1].The genotyping of CSN3 B allele was achieved by means of Hinf I PCR-RFLP[5]

Indicating with N the other alleles at CSN1S1 and CSN3 loci, the genotype distribution of investigated population was 74N/N, 5N/G, 0G/G for CSN1S1 with a G allele frequency of 0.03 and 25N/N, 33N/B, 21B/B for CSN3 with a B allele frequency of 0.47

It can be assumed that the evidenced genetic variability at CSN3 locus for the agerolese cattle genetic type plays an important role in the determination of some fundamental parameters, both from the nutritional point of view that technological, for the production and quality of milk and of DOP products derived from it as the historians cheeses "Provolone del Monaco" and "Fior di latte"

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PHENOTYPING AND EVALUATION OF HEAT STRESS BY THERMAL IMAGING IN THE MANAGEMENT OF MICE.

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The objective of this study was to monitor with thermal imaging the surface temperature of mice within the first 5 days of life, to highlight any significant differences in skin temperature between the heterozygous and homozygous mice.
The subjects will then be monitored with thermographic technique until weaning (21st day of age) to analyse the evolution of body temperature during the development of the nest and the acquisition of full thermoregulatory capacity.[1-2]

The experimental phase, lasting three months, was held at the Institute of Pharmacological Research "Mario Negri" in Milan.
In the SPF facility, the temperature and relative humidity are kept constant (22 ± 2°C, 55 ± 10%), the lighting is set to 12 hours of light and 12 hours of dark(7:00 to 19:00), the air is filtered with HEPA filters, and each object is autoclaved or Peroxide to ensure the SPF status of the animal. Were used:
Cages: No.3 IVC ventilated under positive pressure, air changes at 75 hour.
Mice: n°3 female Hsd: Nude-Foxn1nu athymic (nu / +) of 6 weeks of age.
n° 3 males Hsd: athymic Nude-Foxn1nu (nu / nu) of 6 weeks of age.
Permissions: Ministerial Committee for experimentation.
Litter: corn in cob autoclaved, 150g per cage. The bedding is changed every 2 weeks.
Food: Harlan Teklad Global Diet 2018S reproduction/maintenance. Feeding ad libitum.
Water: autoclaved. Administration in bottles ad libitum.
Laminar Flow Hood: CS5 changing station.
Thermocamera: AVIO TVS500 with uncooled microbolometer detector.
Software: GORATEC Thermography Suite.

In the first hours of life the animals disperse more heat despite the successful containment of the nest, and this aspect indicative, assumed as an index of increased vulnerability, has a delicate role in nature for the survival of the species itself. With decreasing temperature, the set of all metabolic processes decrease in intensity. Graphically it would seem that the thermogenic processes are activated only for the first few seconds of removal from the offspring of parents. They appear instead more and more active as the hours passed, as evidenced by the data reported on the subjects "3-5 days".

The thermographic analysis of the early life stages of nude mice has demonstrated in a concrete theoretical concepts typical of younger individuals. It is necessary to develop a new research protocol to improve the data analysis cencerning the different temperature in the nest to demonstrate the changes as regards the growing of animals.

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ELECTRICAL STUNNING OF TURKEYS IN A WATER BATH: EFFECT OF CURRENT INTENSITY ON THE EFFECTIVENESS OF STUNNING AND MEAT QUALITY.

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The new EU Regulation 1099/2009 on the protection of animals during slaughter, reported in Annex I, Tab. 2, the conditions for the stunning of animals. In particular for electrical stunning of turkeys in a water bath is provided the use of 250 mA, at a frequency less than 200 Hz, and 400 mA at a greater frequency. In all cases the exposure must last at least 4 seconds. According to Raj, the minimum current required per bird to ensure a stunning effective can be determined using three criteria: induce of epileptiform activity, abolition of somatosensory evoked potentials (SEPs) and induction of cardiac arrest (1). It was seen that at a given frequency (50 Hz) is possible to achieve an amperage that inducing cardiac arrest at stunning in all animals (2,3) and although this condition is not a prerequisite for a good stunning, nevertheless has welfare advantages.
However, it has been shown that low frequencies decreases the quality of carcass, in particular of breast muscle (3,4), while the effect of an increase of intensity does not appear to have been demonstrated.
Then we wanted to investigate whether the parameters set by the Regulation, as are applied at turkey slaughter plants (i.e. using high-frequency current and 400 mA intensity), allows to obtain the best guarantees of animal welfare while maintaining a good quality of meat.

To do this, we carried out the tests at a commercial processing plant on 1350 turkeys hens, BIG 6 line of 9.6 kg live weight, dividing them into 3 groups. All groups were exposed to a current of 500 Hz but to variable intensities, respectively 200, 250 and 400 mA, the exposure time was 13 seconds in groups 1 and 2 and 4 seconds for the Regulatory group. Subsequently we evaluated the quality of stunning by animal-based physical indicators (5) and the incidence of defects of meat slicer.

All animals showed signs of unconsciousness but with increasing intensity increased, although not significantly, the percentage of animals that experienced cardiac arrest without signs of recovery after stunning, in particular groups at 250 and 400 mA showed overlapping percentages, respectively 97% and 96%; while the remaining animals showed signs of recovery of consciousness after an average period variable but again comparable between groups at 250 and 400 mA.
From the point of view of carcass, instead, there was a significant difference between the incidence of defects in the group at 400 mA compared to those at lower intensities.

This preliminary work suggests that during the electrical stunning of the turkey it is possible to use parameters less restrictive than those dictated by EU Regulation 1099/2009 while respecting of animal welfare.

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